Docket No.: 066852-0092

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Palsson, Bernhard	Customer No.: 41552
Appl. No.	:	09/923,870	Confirmation No.: 1729
Filed	:	August 6, 2001	
Title	:	METHODS FOR IDENTIFYING DRUG TARGETS BASED ON GENOMIC SEQUENCE DATA	
Grp./A.U.	:	1631	
Examiner:	:	Allen, Marianne P.	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Shankar Subramaniam, Ph.D., declare as follows:

1) I am currently a Professor at the Departments of Bioengineering, Chemistry and Biochemistry at the University of California, San Diego. I was a Professor in the Departments of Physiology, Biochemistry, Chemical Engineering and Electrical and Computer Engineering at the University of Illinois prior to moving to UC San Diego. Previously, I have held Assistant and Associate Professorships at the University of Illinois.

2) I obtained a Bachelors of Science majoring in Chemistry, Physics and Mathematics in 1972 from Osmania University, Master of Science in Chemistry in 1974 from Osmania University and a Ph.D. in Chemistry from the Indian Institute of Technology Kanpur in 1982. I have authored or co-authored numerous papers in the areas of genomics and bioinformatics. My curriculum vitae and list of publications is attached hereto as Exhibit 1.

3) I have reviewed sections pertinent to my below attestation of the above-identified patent application. Specifically, I understand that the application describes and claims computer methods for producing a genome specific stoichiometric matrix and computer methods for

producing an *in silico* representation of a microbe. The methods rely, in part, on the assignment of function to a gene or open reading frame based on nucleotide or amino acid sequence homology.

4) I have read the Office Action mailed June 18, 2004, issued in connection with the above-identified application. I understand that the claims have been rejected, in part, because it is alleged that the application does not describe percent homology criteria for functional categorization of open reading frames based on sequence homology and that the application does not describe criteria for inclusion or exclusion of a gene or open reading frame as a metabolic gene based on sequence homology. While all open reading frames in a genome cannot be automatically assigned function, it was well established at the time the priority application was filed on February 2, 1999, that those open reading frames that have appropriate sequence homology with genes from other microbes whose function was previously assigned could be characterized to possess the same function. While the degree of sequence homology is arguably subjective, databases such as COG (Complete Groups of Orthologous Genes, National Institutes of Health Website that uses bi-directional BLAST; http://www.ncbi.nlm.nih.gov/COG/; *Science* 1997 Oct 24;278(5338):631-37) have criteria that are routinely deployed for function identification through homology.

5) My understanding from reading the application is that, at the time of the priority application, functional assignment through homology was a routine procedure for open reading frames that display homology, and it is a superfluous need for the procedure described in the application to be set forth in more detail than that currently specified. Further, once function identification is made it is common place to characterize the function as pertaining to metabolism from legacy knowledge of cellular metabolism.

6) For example, the application describes that following identification of an open reading frame, well established algorithms can be used to determine the extent of similarity between a given sequence and a gene/protein sequence deposited in the worldwide genetic databases. These algorithms are described at page 7, second paragraph, and include the BLAST and FAST family of programs.

7) The application also describes at, for example, page 7, second paragraph, that if a coding region from a gene is homologous to a gene within one of the sequence databases, the open reading frame is assigned a function similar to the homologously matched gene.

8) The application further describes at, for example, pages 13-14 in Example 1, that the genetic sequence and open reading frame identifications and assignments are readily available for a majority of the *E. coli* genome from a number of on-line locations, citing to both the TIGR and the *E. coli* Genome Project web sites for obtaining this information. Genome annotation data obtained from the latter site was used to obtain those genes involved in cellular metabolism.

9) Based on the above descriptions, it is clear that for this aspect of the claims, all that is necessary with respect to assigning function to encoded proteins is to perform a sequence homology search against a gene or protein sequence database using well known algorithms or programs such as BLAST or FAST to identify if a coding region is homologous to a known gene within a sequence database. Identification of a homologous sequence assigns the protein function of the known homologous sequence to the protein encoded by the query coding region sequence. Accordingly, a positive sequence homology hit to a known sequence of known function results in the assignment of the known function to the query coding region.

10) Based on the above descriptions, it also is clear that in order to practice this aspect of the invention as claimed, all that is necessary with respect to determining if the assigned function of an encoded protein is involved in cellular metabolism is to select those open reading frames that have been assigned a function involved in metabolism. As indicated at pages 13-14, the genome annotation data of the known gene will, in many cases, already contain such an assignment. For example, the annotation data can indicate that a particular gene is involved in glycolysis, one of many well known metabolic processes. In cases where the annotation of a known gene specifies only the name of the protein or a biochemical reaction, published literature can provide information on functional association of the gene with metabolism.

11) Further, at the time the priority application was filed, any experimentation necessary to obtain a sequence homology search and assign a function based on homology to a known gene was predictable and routine. For example, the BLAST and FAST family of sequence alignment algorithms were well known and accepted standards in the art. The percent homology results produced from these programs are based on alignment criteria selected by the user. It is also common to use an E-value that seeks if the two sequences are similar by random chance. It is routinely accepted in the protein research area, that sequences that are likely to be homologous by random chance by one in a tenth of a million are likely to be orthologous or paralogous sequences. Accordingly, the predictability of selection and assignment of function based on homology to a known gene is set by the user and can be based on well established criteria.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Date: Dec 20, 04

By: Shankar Subramaniam
Shankar Subramaniam, Ph.D.

CURRICULUM VITAE (SHORT VERSION)

SHANKAR SUBRAMANIAM

RESEARCH PROFILE:

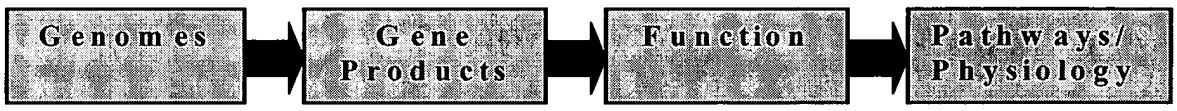
Shankar Subramaniam is a Professor of Bioengineering, Chemistry and Biochemistry and Biology and Director of the Bioinformatics Graduate Program at the University of California at San Diego. He also has adjunct Professorships at the Salk Institute for Biological Studies and the San Diego Supercomputer Center. He is also a Guest Professor at the Center for Molecular Biology and Neuroscience at the University of Oslo in Norway and Professor at the Center for Cardiovascular Bioinformatics and Modeling at Johns Hopkins University. He is a fellow of the American Institute for Medical and Biological Engineering (AIMBE) and is a recipient of Smithsonian Foundation and Association of Laboratory Automation Awards and his research work is described below. Subramaniam was honored in 2002 as "High Performance Computing's Highest Guru" by Genome Technology Magazine. He is on the Editorial Board of the following Journals: Bioinformatics, Journal of Molecular Recognition and the journal, Molecular and Cellular Proteomics. He is on several NIH advisory committees and panels. His research spans diverse areas of bioinformatics and systems biology.

RESEARCH INTERESTS OVERVIEW

The sequencing of several mammalian genomes provides a basis for understanding the systemic functioning of living cells. The "omics" technologies have begun to produce vast amounts of context-specific biological data

Thus, future developments in genomics, and the applications that derive from genomics, will be dependent upon the scientific progress at the interface of three major disciplines; biology, engineering, and computer science. My laboratory works in this interdisciplinary areas of Bioinformatics and Systems Biology.

Bioinformatics characterizes the flow of information in living systems. This is schematically represented below.



Specific projects our laboratory is associated with in this area include,

Genome Annotation

1. Design of a novel algorithm for generating protein family specific scoring matrices and applications of this scoring scheme in identifying orthologous and paralogous proteins in genomes.
2. Design of nucleotide and protein profiles that will serve to develop a high throughput method to identifying genes in genome sequences. This has implications for expression

profiling technology using oligonucleotides for assessing cell function.
3. Design and development of high throughput pipeline for annotating genomes.

Protein Sequence-Structure Mapping

1. Development of strategies for obtaining unique protein fragment structures that will serve as the basis set for modeling three-dimensional structures of all soluble proteins.
2. Characterizing protein folds using fragment-based methods.
3. Obtaining statistical potentials (pairwise atomic PDFs) for defining energy landscape of proteins.
4. Using global optimization strategies for modeling structures of proteins from their primary sequences.

Functional Genomics

1. Development of new methods for gene expression analysis.
2. Reconstruction and biochemical analysis of transcriptional networks.
3. Functional analysis of transcription factors.

Reconstruction and Modeling of Biochemical Pathways

1. Reconstruction of disease networks pertaining to insulin resistance.
2. Design and development of methods for analyzing cell signaling pathways from expression profile and proteomic data.
3. Identification of protein partners in cell signaling pathways.
4. Computational modeling of pathways.

Infrastructure for Biological Databases, Analysis Tools and Interfaces

1. Extensions of the Biology Workbench (http://workbench.sdsc.edu) developed in my laboratory.
2. Design of novel tools for expression profile and proteomic analysis in the Workbench.
3. Creation of automated pipelines for genome, gene and protein analysis.
4. Design and development of Laboratory Information Management Systems.
5. Development of the Information Management System for Alliance for Cellular Signaling and LipidMaps projects.

http://genome.ucsd.edu/

http://www.signaling-gateway.org/

http://www.lipidmaps.org/

http:// www.mitorproteome.org/

http://www.bioinformatics.ucsd.edu/

Funding Acknowledgement: NSF, NIH, State of California and Corporate

AFFILIATIONS:

Professor
Department of Bioengineering
Department of Chemistry & Biochemistry
San Diego Supercomputing Center
Salk Institute
Director
Bioinformatics Program, UCSD
Guest Professor
Center for Molecular Biology and Neuroscience
University of Oslo, Norway

EDUCATION:

B.Sc., (Chemistry) Osmania University 1972 (Recipient of gold medal for ranking first in the University)
M.Sc., (Physical Organic Chemistry) Osmania University 1974 (First Class with distinction)
Ph.D., (Chemistry) Indian Institute of Technology Kanpur 1982

PROFESSIONAL EXPERIENCE:

1999-present	Professor, Departments of Bioengineering, Chemistry and Biochemistry, Director, Bioinformatics Graduate Program University of California San Diego and Senior Fellow, San Diego Super Computer Center.
1998-1999	Professor, Departments of Biochemistry, Molecular and Integrative Physiology, Chemical, Electrical and Computer Engineering, Center for Biophysics and Computational Biology, Beckman Institute and National Center for Supercomputing Applications. University of Illinois at Urbana-Champaign.
1996-1997	Associate Professor, Department of Molecular and Integrative Physiology, Department of Chemical Engineering, Center for Biophysics and Computational Biology, Beckman Institute and National Center for Supercomputing Applications. University of Illinois at Urbana-Champaign.
1991-1996	Assistant Professor, Department of Molecular and Integrative Physiology, Center for Biophysics and Computational Biology, Beckman Institute and National Center for Supercomputing Applications. University of Illinois at Urbana-Champaign.
1990-1990	Visiting Scientist, Princeton University and Senior Research Scientist, Squibb Institute for Medical Reseach.
1986- 1989	Assistant Director for Scientific Development, IMD and Visiting Assistant Professor of Chemistry, University of Houston. Work carried out in the group of Professor J. Andrew McCammon
1985-1986	Postdoctoral Research Associate, University of North Carolina, Chapel Hill. Work carried out in collaboration with Profs. Jan Hermans and Max Berkowitz.
1984-1985	Postdoctoral Research Associate, University of North Carolina, Chapel Hill. Work carried out in the group of Prof. Robert G. Parr.
1982-1984	Lecturer in Chemistry, St. Stephen's College, Delhi University.
1979-1982	Senior Research Assistant, Indian Institute of Technology Kanpur.
1976-1979	Research Assistant, Indian Institute of Technology Kanpur.

HONORS:

Genome Technology All Star Award, 2002
Association of LabAutomation Award, 2001
Elected Fellow, Institute for Biomedical Engineering, 2000
The College of Engineering, University of Illinois Advisor's List, 1997.
Smithsonian Foundation Citation for Innovation in Computing, 1997.
Council of Scientific and Industrial Research Fellowship 1974-1976.
Indian National Merit Scholarship 1972-1974.
Thatte memorial gold medal in Bachelor's degree 1972.

PROFESSIONAL ACTIVITIES (National Committees and Forums): (Partial List, 1997-03 only)

1. Member, National Academies of Sciences Future of Supercomputing Committee, 2003-2004.
2. Volume Editor, Wiley Encyclopedia of Genomics, Genetics, Proteomics and Bioinformatics. 2004.
3. Member, Scientific Advisory Panel, NURSA project, NIDDK, NIH. 2004-present.
4. Member, NIH Director's Roadmap Committee for Bioinformatics, 2003
5. Chair, Software Maintenance and Development Study Section, NIH, 2002- present
6. Chair, San Diego Supercomputer Center Executive Committee, 2002 - present
7. Guest Professor, Centre for Molecular Biology and Neuroscience, University of Oslo, Norway 2002 – present
8. Editorial Board Member, Bioinformatics. 2002-present.
9. Editorial Board Member. Molecular and Cellular Proteomics 2002-present.
10. Editorial Board Member. Journal of Molecular Recognition. 2000-present.
11. Editor, Wiley Encyclopedia on Genomics, Proteomics and Bioinformatics 2002 - present
12. Member, Search Committee for Director of Bioinformatics and Computational Biology Institute, NIGMS, NIH, 2001
13. Chair, BISTI Study Section, NIH 2001-2002
14. Chair, NIH/NSF Bioengineering and Bioinformatics Education and Training Workshop 2001
15. US Representative in the Global Science Forum Neuroinformatics Working Group 2000-present
16. Member, Scientific Advisory Board to the Department of Bioengineering, UI Chicago 2000-present
17. Member, Scientific Advisory Board, Mitokor Corporation, 2001-present
18. Member, Scientific Advisory Board, Genomar, 1999-present
19. Member, SUN Microsystems Informatics Advisory Council 2000-present
20. Member, Review Panel, NSERC, Berkeley 1999-present
21. Member, Advisory committee to the Working Group of the Director of NIH, on Bioinformatics (Chairs: Botstein/Smarr) 1998-99
22. Member, NIH Site Visit Panel on Program Project Grant, Lawrence Berkeley Laboratory, 1999.
23. Member, Whitaker Foundation Workshop on Bioengineering Education. 1999
24. Invited Participant, AIChe Planning Group in Bioinformatics, 1999.
25. Adhoc Member, Genetics Study Section, NIH, 1998
26. Panelist, Advisory Panel on Postdoctoral Fellowships in Bioinformatics of the National Science Foundation, 1999-2000
27. Panelist, Committee on Knowledge and Distributed Intelligence Initiative, NSF 1998

28. Member, NIH Review Panel on Workshop and Training Grants in Computational Genomics and Bioinformatics, 1999.
29. Chair, Session on Computational Biophysics Session, Annual Meeting of the Biophysical Society, 1999.
30. Proposal Reviewer, W.M. Keck Foundation, 1998
31. Member, Scientific Advisory Board, BioSoft Inc. Norway 1998-present
32. Member, Advisory Committee, Fralin Biotechnology Center, Virginia Tech. 1998-99
33. Chair & Organizer, Beckman Institute Symposia on Protein Structure, Function and Bioinformatics, 1991-97.
34. Member, Editorial Board for Journal of Molecular Recognition, 1999-present
35. Ad-Hoc Member, SBIR/STTR Study Section, NIH, 1997-98.
36. Member of the Working Group of the Advisory Committee to the Director of NIH on Biological Information Science and Technology Initiative. Participated in writing the BISTI report to NIH (1997-1999)

INVITED LECTURES (Partial List 1991-1999):

1. International Symposium on Polymer Modeling, NCSA. "Computer Simulations of Biomolecules". May 1991.
2. Department of Chemistry, Northern Illinois University, DeKalb. "Molecular Recognition in Proteins". Oct. 1991.
3. Eli Lilly Laboratories, Indianopolis. "Protein Structure Modeling". Sept. 1991.
4. Iowa Academy of Sciences, Cedar Falls. "Supercomputing in Biology". Nov. 1991.
5. Fourth International meeting on Software Engineering and Knowledge Engineering, Capri, Italy . "Knowledge-Based Approaches to Protein Structure and Motifs". June 1992.
6. Neural Networks - Biology to High Energy Physics, Elba, Italy. "Machine Learning Approaches to Protein Feature Prediction". June 1992.
7. Department of Chemical Engineering, Illinois. "Computer Simulations of Biomolecular Recognition". Sept. 1992.
8. Pfizer Central Research Laboratories. "Biomolecular Recognition", and " Protein Structure Prediction from Sequence". Sept. 1993.
9. Center for Advanced Research in Biotechnology, University of Maryland. "Electrostatics and Molecular Recognition". Oct. 1993.
10. International Symposium on Distance-based Approaches to Protein Structure, Lyngby, Denmark. Plenary Lecture on "Protein Structure Prediction - Past and Present". Nov. 1993.
11. North Dakota State University, Fargo. "Protein Structure and Modeling". April 1994.
12. 1994 First World Congress on Computational Medicine, Health and Biotechnology. Session Chair and Speaker. "Knowledge-Based Approaches to Protein Structure". April 1994.
13. Pfizer-Beckman Symposium on Protein Structure and Engineering, University of Illinois. "Knowledge-Based Tools for Protein Engineering and Design". June 1994.
14. Antibody Workshop, Cambridge, U.K. "Kinetics and Energetics in Immune Recognition". Aug. 1994.
15. Short Course on "Protein Modeling" in SISSA, Trieste, Italy. Sept. 1994.
16. Texas A & M University, College Station. "Knowledge-Based Approaches to Protein Structure", Dec 1994.

17. University of California at Irvine. "Molecular Recognition in Proteins" - From Lock and Key to Float, Fix, Fit and Fasten., May 1995.
18. ISIS Pharmaceuticals, San Diego, CA. "Protein Structure Prediction - Novel Methods"., May 1995.
19. Intelligent Systems for Molecular Biology-95. Tutorial titled "Molecular Biology for the Computer Scientist". Cambridge, U.K. July 1995.
20. Brandeis University, Dept. of Chemistry. "Knowledge-based Potentials for Proteins". Sept. 1995.
21. Istituto Ricerca BioMoleculare P. Angeletti, Pomezia. "Protein Structure Prediction Methods". Nov. 1995.
22. Centro Nazionale Ricerca (CNR) Genoa. "Simulations of Biological Membranes". Nov. 1995.
23. Rush Medical School, Chicago, IL. "Protein Structure and Folding". Jan. 1996.
24. Univeristy of California at Irvine, "Protein Folding in machino". March. 1996.
25. Purdue University, IN., "Knowledge-Based Methods for Protein Structure". March 1996.
26. University of Wisconsin at Madison, WI. "Molecular Recognition - Through a Computational Microscope". March 1996.
27. International Center for Theoretical Physics, Trieste, Italy. "Methods for Protein Structure and Folding", March 1996.
28. International Center for Theoretical Physics, Trieste, Italy. "Computer Simulation Methods for Molecular Recognition", March 1996.
29. Eli Lilly and Company, Indianapolis. "The Biology Workbench ". April, 1996.
30. ISMB96, Talk on "Knowledge-based Methods for Protein Structure Refinement and Prediction". June 1996.
31. ISMB96, Presentation and National Release of the "Biology Workbench". June 1996.
32. University of Minnesota, MN., "Protein Folding *in machino*" . Oct., 1996.
33. University of Minnesota, MN., "Biology Workbench - A Seamless Integration of Dabatases, Analysis Algorithms and Interfaces on the World Wide Web". Oct. 1996.
34. ASBMB Conference on Computational Biology - Methods in Biomolecular Imaging. "A Knowledge-Based Method for Protein Structure Refinement". Oct., 1996.
35. USDA International Symposium on "Genetic Analysis of Economically Important Traits in Livestock" , Allerton, Illinois. "BioInformatics on the World Wide Web". Nov., 1996.
36. Chemical Engineering Symposium on Engineering Protein Recognition, Univ. of Illinois at Urbana-Champaign. "Role of Electrostatic Interactions in Antigen-Antibody Interactions". November, 1996.
37. Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC. Workshop on Protein Folding. December, 1996.
38. Midwest Regional ACS Meeting., Midland, MI., Bioinformatics on the Web., May 1997.
39. NSF Workshop on "Knowledge Network Processing, U. Pennsylvania., June 1997.
40. Second International Conference on Molecular Recognition on Surfaces, Basel, Switzerland, July 1997.
41. Department of Chemical Engineering, University of Wisconsin at Madison, Bioinformatics and the Future., Sept. 1997.
42. 55.Departments of Physics & Biochemistry, Oklahoma State University, Stillwater. Protein Folding i*n machino*, Oct. 1997.
43. Genome Workshop on Databases, "Bioinformatics and Genomics", Minnesuing Acres, WI, Oct. 1997.
44. Department of Biophysics, Johns Hopkins University, Protein Folding i*n machino*, Dec. 1997.
45. National Institutes of Health BECON Symposium Panel on "Bioinformatics", Feb. 1997.

46. Institute for Biomedical Computing, Washington University, St. Louis, Protein Structure and Folding, March 1998.
47. Departments of Computer Science and Biology, University of Kentucky, The Biology Workbench – A paradigm for virtual computing on the web, April 1998.
48. Department of Biochemistry, Northern Illinois University, DeKalb. Molecular Recognition in the Immune System, April 1998.
49. Department of Chemistry, Rice University. Protein Structure and Folding, April 1998.
50. Department of Bioengineering, UC San Diego, La Jolla. Bioinformatics – The Rosetta Stone of Biology, June 1998.
51. Department of Bioengineering, UC San Diego, La Jolla. Molecular Recognition in Proteins, August 1998.
52. European Molecular Biology Organization, "Talking Proteins Symposium", Heidelberg, Sept., 1998.
53. Department of Computer Science, Purdue University, Bioinformatics., Oct., 1998.
54. Department of Bioengineering, Rice University, Houston, Bioinformatics in the Postgenome Era, Nov., 1998.
55. Chem. Eng. Dept. Massachusetts Institute of Technology, Bioinformatics: Deciphering the Rosetta Stone of Biology, April, 1999
56. Chemistry Dept., Wabash College, Bioinformatics, April 1999
57. Physiology & Biophysics, Cornell Medical School, Applications of Bioinformatics, NY, May 1999

SINCE JOINING UCSD

58. Symposium on "Bioinformatics, Patents and Intellectual Property Rights" organized by McDermott and Campbell & Flores Law Firms, La Jolla, San Diego, June 1999.
59. Annual Symposium on Biomedical Engineering, "Bioinformatics in the Post-genome Era", Salt Lake City, Utah, July 1999.
60. Chattaqua Conference on Computational Science, "Bioinformatics", Boston University, Sept. 1999.
61. Department of Chemistry at UNC Chapel Hill, "Deconstructing Protein Structure", Oct., 1999.
62. Department of Chemistry and Biochemistry, Duke University, "Bioinformatics – Deciphering the Rosetta Stone of Biology", Oct., 1999.
63. BMES Annual Symposium. Atlanta, GA, "Postgenomic Bioinformatics", Oct. 1999.
64. Chair, Panel Discussion on Bioinformatics, BMES Annual Meeting, Atlanta, GA, Oct. 1999.
65. 3rd TIGR Symposium on Computational Genomics, Baltimore, MD., "Sequence-Function Mapping – Lessons from Bioinformatics" , Nov., 1999.
66. Department of Computer Science, San Diego State University, "Postgenomic Bioinformatics – Computer Science Challenges", Nov., 1999.
67. CSU Biotechnology Symposium, Keynote Lecture, "Bioinformatics: Deciphering the Rosetta Stone of Biology", CalPolytechnic Pomona, Jan., 2000.
68. BioStar Meeting on Bioinformatics, Bioinformatics: challenges and progress, UC San Diego., Jan 2000.
69. Pacific Rim Society Internation Symposium on Bioinformatics. Bioinformatics at the Crossroads of Biology, Medicine and Information Science. Los Angeles, March, 2000.
70. 19th Southern Biomedical Conference, Keynote Lecture. The future of Bioinformatics., Blacksburg, Virginia, April 2000.
71. Genetic Resources for the New Century, Beyond the Genome: Bioinformatics. San Diego Zoo., May 2000.

72. Global Forum on Neuroinformatics organized by National Institutes of Mental Health, "Design of a Neuroinformatics Workbench", Genoa, Italy, May 2000.
73. Massachusetts Institute of Technology Workshop on Bioinformatics. "Protein Structure and Folding (4 Lectures)., MIT, Boston, June 2000.
74. NIH Site Visit to evaluate a Program Project Grant, New Jersey Institute of Technology, June 2000.
75. Iceland Genome Project. "Bioinformatics after the Genome" Reykjavik, July, 2000.
76. International Society for Animal Genetics Conference, Keynote Lecture: "Bioinformatics: Mapping the New Central Dogma in Biology", Minneapolis, July 2000.
77. Keynote Speaker, "The cross-education across biology and information science", Conference in Bioinformatics Education in Undergraduate Curricula. San Diego State University. July 2000.
78. Mitokor Corporation, Designing Infrastructure for Bioinformatics. San Diego, August 2000.
79. Keynote Speaker, EUCHEM conference on BCDC- Bioinformatics, Cheminformatics, Datamining, MQSAR and Chemometrics, Stockholm, Sweden. Bioinformatics. Swedish Chemical Society, Stockholm. Septermber 2000.
80. Mt. Sinai Medical School, Department of Pharmacology. Bioinformatics: Applications to sequence and function analysis. New York, October 2000.
81. Conference, Metabolic Engineering III, Sequence-structure-function mapping for engineering microorganisms. Colorado Springs, October 2000.
82. SUN Microsystems. Bioinformatics: Challenges for Software, Database and Computer Designers. Palo Alto, November 2000.
83. PacificChem. Chair and Speaker, Bioinformatics Session. Beyond the Genome. December 2000.
84. 5th Lake Tahoe Symposium on Molecular Diversity. Introduction to the Alliance for Cellular Signaling. Lake Tahoe, January 2001.
85. Keynote and Award Lecture. LabAutomation 2001. Bioinformatics: At the Crossroads of Biology, Medicine and Engineering. Palm Springs. January 2001.
86. UCSD Chemistry-Industry Presentation. Challenges in Post-Genome Bioinformatics. UCSD. February 2001.
87. Health Care in the New Millenium: Merging Biology, Information Technology and Engineering, organized by AIMBE. The Information Revolution in Biology and its Impact on Medicine in the New Millenium. National Academy of Sciences. Washington D.C. March 2001.
88. California State University Pomona, Departments of Chemistry and Biochemistry. Bioinformatics: Flow of Information in Biology. March 2001.
89. BMES-FASEB Meeting. Chair and Speaker. Bioinformatics Session. Orlando April 2001.
90. Institute for Theoretical Physics, UC Santa Barbara. Modeling biological processes. April 2001.
91. Department of Bioengineering. UI Chicago. Advisory Committee Meeting. April 2001.
92. Plenary Lecture. Symposium of European Society for Engineering and Medicine. Bioinformatics: The Interface between Biology, Engineering and Medicine. Belfast, Ireland. May 2001.
93. Bioinformatics Course in MIT Workshop in Bioinformatics. MIT, Boston. June 2001.
94. Databasing the Brain. University of Oslo and European Commission and Global Science Forum. Chair and Speaker. Bioinformatics and Neuroinformatics: Challenges and Opportunities. Oslo, Norway. July 2001.
95. Salk Institute. Bioinformatics of Cellular Signaling. La Jolla. July 2001.

96. Gordon Research Conference on Bioinformatics- From Inference to Predictive Models. Designing Knowledge Bases for Cellular Signaling. Tilton School, New Hampshire, August 2001.
97. Aspen Workshop on Enzymatic Networks and Cellular Signaling. Data Modeling and Ontologies for AfCS. Aspen, August 2001.
98. Computational Challenges in the Post-Genomic Age II. Databases for Modern Biology. Keynote Lecture. Research Triangle Park, North Carolina. September, 2001.
99. The O'Reilly Bioinformatics Technology Conference. State of the Art in Integrative Bioinformatics. Section Speaker. Tucson, Arizona. January 2002.
100. Biophysical Society Annual Meeting. Semiotics of Cellular Signaling. San Francisco, February 2002
101. University of Minnesota Bioinformatics Symposium. Challenges in Integrative Bioinformatics. Minneapolis, Minnesota, April 2002.
102. In Silico Biology Conference. Molecular Ontology for Cellular Signaling. San Diego, June 2002.
103. University of Utah Department of Medical Informatics. Postgenome Bioinformatics: At the Crossroads of Biology, Engineering and Medicine. September 2002.
104. SIAM. Structuring Cellular Data for Computational Modeling. Washington DC, October 2002.
105. La Jolla Mesa Signaling Group. AFCS Molecule Pages. La Jolla, October 2002
106. Oracle Life Sciences Conference. Structuring Biological Data. San Francisco, November 2002
107. International Business Forum. Investing and Partnering in Systems Biology. San Francisco, November 2002
108. Harvard-MIT Seminar in Computational Biology. Challenges in Post Sequence Bioinformatics and Systems Biology. Boston, November 2002.
109. Mount Sinai School of Medicine. Modeling Cellular Complexity. New York City, December 2002.
110. New York Academy of Sciences Conference. Understanding the Cell: Components, Networks and Modeling. New York City, December 2002.
111. IBM Academy of Technology. Challenges in Post-Sequence Bioinformatics and Systems Biology. San Jose, California, March 2003
112. Knowledge Millennium III: The Business of Biotechnology. New Delhi, India
113. Purdue University. Systems Biology: Windows into Intracellular Networks. Lafayette, April 2003.
114. Purdue University. Systems Biology: Challenges in the Post Genome Era: The Biology-Computational Science Interface. Lafayette, April 2003.
115. Virginia Bioinformatics Institute. Systems Biology: Challenges in the Post-Genomic Era. Bethesda, May 2003
116. NIH Human Brain Project. Postgenomic Biology: Deciphering Intracellular Networks. Bethesda, May 2003
117. Salk Institute. Deciphering Intracellular Networks in Mammalian Cells. La Jolla, August 2003.
118. Gordon Research Conference. Reconstruction and Modeling of Networks from Cellular Data. Queen's College, Oxford, August 2003.
119. Johns Hopkins University, CCBM Inaugural Seminar, "Deciphering Intracellular Networks in Mammalian Cells". Baltimore, September 2003.
120. CalIT^2. Systems Biology, Bioengineering and Bioinformatics: A new discipline at the Interface of Biology, Medicine and Information Technology. La Jolla, September 2003.
121. Pathways, Networks and Systems Biology Conference. Deciphering Intracellular Networks in Mammalian Cells. Santorini, Greece, September 2003.

122. Scientific Data Integration: Challenges and Some Solutions. Biomedical Information Science and Technology Initiative Consortium. Bethesda, MD, November 2003.
123. 2003 PKR Protein Phosphorylation Workshop. .Alliance for Cellular Signaling: Design of the Informatics Infrastructure. Asilomar, CA December 2003.
124. IPAM: Proteomics Workshop I: High Throughput Technologies and Methods of Analysis. "Reconstruction of Cellular Networks: the Biological Context". Los Angeles, March 2004.
125. NHLBI: A Systems Biology Approach to Regulatory Networks in Heart, Lung, Blood and Sleep Research Working Group." Biological Data Integration and Analysis: Challenges for Mammalian Cell Systems. Bethesda, MD, May 2004.
126. Massachusetts Institute of Technology: MIT Summer Course, "Bioinformatics: Principles, Methods and Applications" (five lectures). Boston MA, June 2004
127. Stazione Zoologica: "Deciphering Mammalian Intracellular Networks: Integrative Data Analysis". Naples, Italy, July 2004.
128. Iowa State University: "Cellular Systems Biology: Data Integration, Analysis and Modeling". Ames IA, July 2004
129. University of Oslo: "Ontologies, Databases and Tools for Brain Imaging. Research Course. Oslo Norway. August 2004.
130. IEEE-EMBC 04 Plenary Lecture: "Bioinformatics and Computational Systems Biology: At the Cross Roads of Biology, Engineering and Computation". San Francisco CA, September 2004.
131. Science & Technology Expert Partnership (STEP) program, Conference on Computational Biology: "Data Integration and Analysis for the Reconstruction of Mammalian Cellular Networks". Washington DC, September 2004.
132. Plenary Lecture, Frontiers in Oncology and Pathology Informatics, "Decoding networks in mammalian cells", Pittsburgh, October 2004.
133. Special Lecture at the Nanotechnology Center, Purdue University, "Bioinformatics and Systems Biology: At the interface of biology, engineering and computer science". Purdue, October 2004.

PUBLICATIONS:

Refereed Publications 2001-2004

1. Avidor-Reiss T, Maer AM, Koundakjian E, Polyanovsky A, Keil T, Subramaniam S, Zuker CS. Decoding cilia function; defining specialized genes required for compartmentalized cilia biogenesis. Cell. 117(4):527-39, 2004.
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6. Giridhar Chukkapalli, Chittibabu Guda and Shankar Subramaniam. SledgeHMMER: a web server for batch searching the Pfam database. Nucleic Acids Research 32, Web Server issue 2004.

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Invited Papers

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4. D. K. Tcheng and S. Subramaniam. Machine Learning Approaches to Protein Feature Prediction in Proceedings of the Workshop, Neural Networks - Biology to High Energy Physics, Elba, Italy, 1992.
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ABSTRACTS AND PRESENTATIONS IN CONFERENCES:

Over 200 Poster and Conference Presentations.

OUTSIDE COMMITTEES AND REVIEW GROUPS:

NIH Software Maintenance Review Panel 2004
NIH NIDDK SAB, NURSA Project 2004
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NIMH Small Working Group on Neuro Ontology 2002
NIH Genetics Study Section Ad-Hoc member 2002
NIH Chair, Special Study Section H 2002
NSF-NIH Panel on Bioinformatics Education 2001
NIH Site Visit Panel 2000
NSF Panel, ITR 2000
NERSC Grant Review Panel 1999- to date
Editorial Board, Journal of Molecular Recognition 1998 – to date
DOE-NSF Panel on "The Future of Computational Biology", 1998
NIH Genetics Study Section Ad-hoc member 1998
Proposal Reviewer, W.M. Keck Foundation, 1998
NIH SBIR-STTR Study Section Ad-hoc member 1998
State of Illinois Biotechnology Planning Committee 1998
NSF Molecular Biophysics Division Reviewer 1997
NIH SBIR-STTR Study Section Ad-hoc member 1997
NIH Site Visit Panel on an NIH Resource Proposal 1997
NSF CISE Panel 1997
NSF Molecular Biophysics Division Reviewer 1997
NRC Molecular Biology Reviewer 1997
Review Panel, SIGGRAPH 1997

SCHOOL, COLLEGE, AND CAMPUS COMMITTEES:

UCSD

Chair, Executive Committee, San Diego Supercomputer Center 2002-present
Member, Technology Directions Committee, UCSD, 2002-present
Member, Campus Advisory Committee on Information Technology, 2000-present
Life Sciences Council, Chair of Education Committee 2002
Member, Research Council, School of Medicine, 2001-present
Director, Graduate Program in Bioinformatics, 2000-present
Chair, Faculty Search Committee, Department of Bioengineering, 2001-2002
Faculty Search Committee, Department of Biology, UCSD 2000
Faculty Search Committee, Department of Chemistry and Biochemistry 1999
Faculty Search Committee, Department of Chemistry and Biochemistry 2000
Faculty Tenuring and Promotion Committee 2000, 2001
Faculty Endowed Chair Selection Committee 2000, 2001
SDSC Executive Committee 2000 – 2002

Pre-UCSD

Member, Biotechnology Council, UIUC 1999
Co-Director, Keck Institute for Comparative and Functional Genomics 1996-1999
Member, Bioengineering Planning Committee, UIUC 1999-1999
School of Molecular and Cellular Biology Strategic Planning Committee, UIUC 1998 – 1999
University of Illinois Biotechnology Planning Committee 1998 – 1999
Beckman Institute Advisory Committee 1998 – 1999
Structural Biology Faculty Search Commiteee 1998
Chair, Center for Biophysics Courses and Curriculum Committee, 1996-99
NCSA Small Allocations Committee 1993 – 2000
Comparative Genomics Faculty Search Committee 1998
Statistical Genetics Faculty Search Committee 1997
Dept. of Molecular and Integrative Physiology Computers Committee, 1994-97
Campus Undergraduate Honors Awards Committee 1995-97
Beckman Instiute Symposium Committee 1991-97

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Extramural Agencies:
DOE Computer Allocation Panel
NSF Panels
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Siggraph 1995, 1996
NIH Study Sections

Reviewer for Journals:
Proceedings of National Academy of Sciences
Biophysical Journal

Physical Review A & B
Metabolic Engineering
Journal of Molecular Biology
Proteins - Structure, Function and Genetics
Biochemistry
Protein Science
J. Computational Biology
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J. Physical Chemistry
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Peng-Liang Wang	2003 - present	
Mano Maurya	2003 – present	

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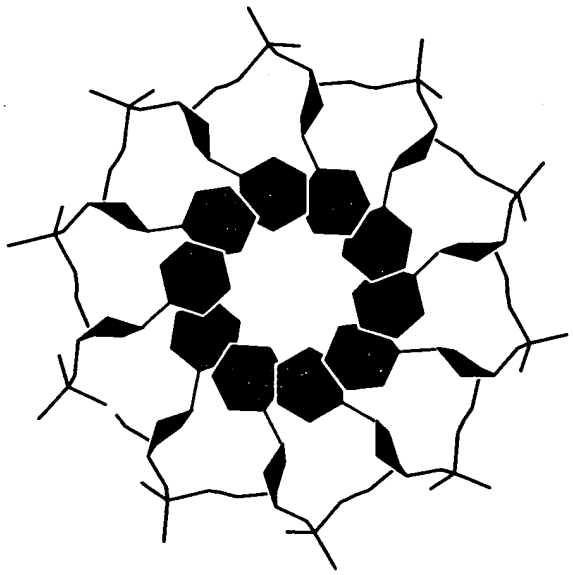

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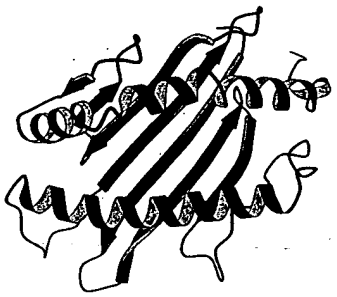
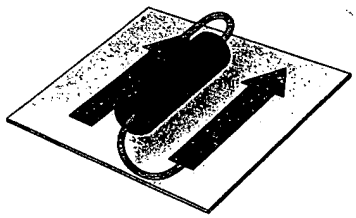
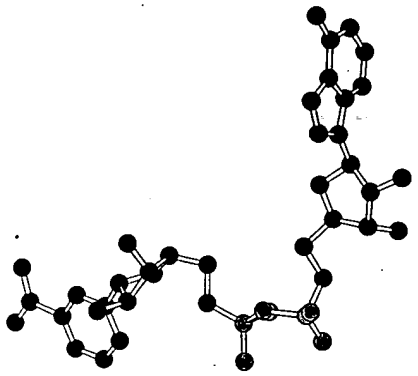
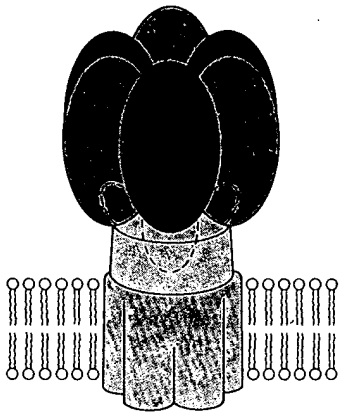
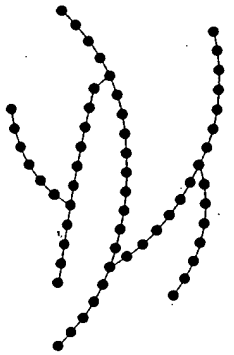
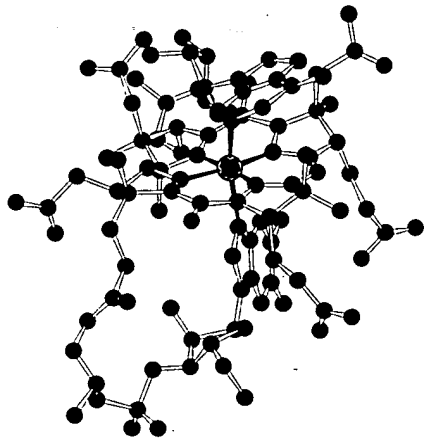


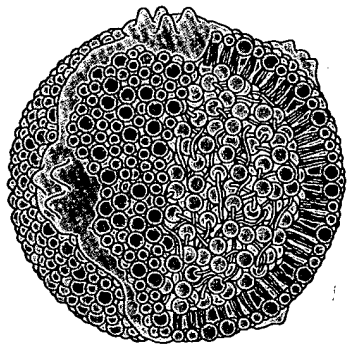












CHAPTER

19

Glycolysis

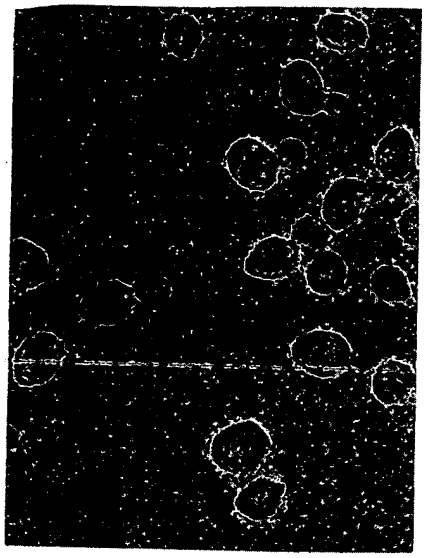
We begin our consideration of the generation of metabolic energy with glycolysis, a nearly universal pathway in biological systems. *Glycolysis is the sequence of reactions that converts glucose into pyruvate with the concomitant production of a relatively small amount of ATP.* In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, which together harvest most of the energy contained in glucose. Under aerobic conditions, pyruvate enters mitochondria, where it is completely oxidized to CO_2 and H_2O. If the supply of oxygen is insufficient, as in actively contracting muscle, pyruvate is converted into lactate. Under anaerobic conditions, yeast transforms pyruvate into ethanol. The formation of ethanol and lactate from glucose are examples of fermentations.

Glycolysis—
Derived from the Greek stem *glyk-*, "sweet," and the word *lysis*, "dissolution."

The elucidation of glycolysis has a rich history. Indeed, the development of biochemistry and the delineation of this central pathway went hand in hand. A key discovery was made by Hans Buchner and Eduard Buchner in 1897, quite by accident. They were interested in manufacturing cell-free extracts of yeast for possible therapeutic use. These extracts had to be preserved without using antiseptics such as phenol, and so they decided to try sucrose, a commonly used preservative in kitchen chemistry. They obtained a startling result: sucrose was rapidly fermented into alcohol by the yeast juice. The significance of this finding was immense. *The Buchners demonstrated for the first time that fermentation could occur outside living cells.* The accepted view of their day, asserted by Louis Pasteur in 1860, was that fermentation is inextricably tied to living cells. The chance

Fermentation—
An ATP-generating process in which organic compounds act as both donors and acceptors of electrons. Fermentation can occur in the absence of O_2. Discovered by Pasteur, who described fermentation as *"la vie sans l'air"* ("life without air").

Opening Image: *Three-dimensional structure of phosphofructokinase, a key enzyme in glycolysis. Fructose 6-phosphate (red), a substrate, is next to ADP (blue), which occupies the ATP-binding site. The bacterial enzyme is allosterically activated by ADP (green) at a distant site. Only one of the four subunits of the tetrameric enzyme is shown here. [Drawn from 4pfk.pdb. T. Schirmer and P.R. Evans. Nature 343(1990):140. The image was generated by Dr. Anthony Nicholls using GRASP, described in A. Nicholls, K. Sharp, and B. Honig. Proteins 11(1991):281.]*



Light micrograph of yeast cells. [Courtesy of Dr. Randy Schekman.]

Enzyme—
A term coined by Friedrich Wilhelm Kühne in 1878 to designate catalytically active substances that had previously been called ferments. Derived from the Greek words *en*, "in," and *zyme*, "leaven."

discovery of the Buchners refuted this vitalistic dogma and opened the door to modern biochemistry. *Metabolism became chemistry.*

The next critical contribution was made by Arthur Harden and William Young in 1905. They added yeast juice to a solution of glucose and found that fermentation started almost immediately but soon ceased unless inorganic phosphate was added. They deduced that *inorganic phosphate became linked to a sugar,* and they proceeded to isolate a hexose diphosphate, which was later shown to be fructose 1,6-bisphosphate, a key intermediate in glycolysis. Furthermore, Harden and Young discovered that yeast juice lost its activity if it was dialyzed or heated to 50°C. However, activity was restored when inactive *dialyzed* juice was mixed with inactive *heated* juice. Thus, activity depended on the presence of two kinds of substances: a heat-labile, nondialyzable component (called *zymase*) and a heat-stable, dialyzable fraction (called *cozymase*). We now know that "zymase" consists of a number of enzymes, whereas "cozymase" consists of metal ions, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and coenzymes such as nicotinamide adenine dinucleotide (NAD^+).

Studies of muscle extracts then showed that many of the reactions of lactic fermentation were the same as those of alcoholic fermentation. *This was an exciting discovery because it revealed an underlying unity in biochemistry.* The complete glycolytic pathway was elucidated by 1940, largely because of the pioneering contributions of Gustav Embden, Otto Meyerhof, Carl Neuberg, Jacob Parnas, Otto Warburg, Gerty Cori, and Carl Cori. Glycolysis is also known as the *Embden-Meyerhof pathway.*

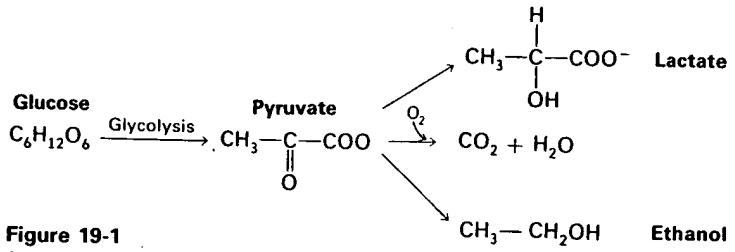
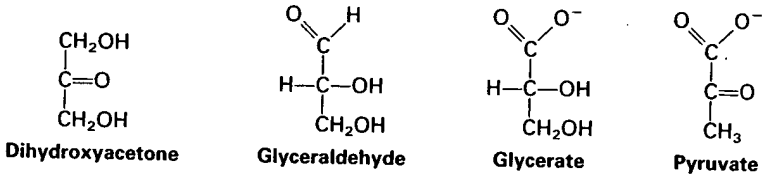
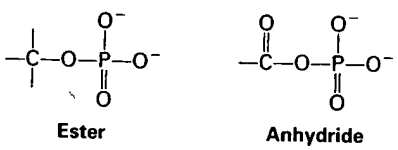


Figure 19-1
Some fates of glucose.

AN OVERVIEW OF KEY STRUCTURES AND REACTIONS

Learning the sequence of events in a metabolic pathway is easier with a firm grasp of the structures of the reactants and an understanding of the types of reactions taking place. Glycolytic intermediates have either six or three carbons. The *six-carbon units* are derivatives of *glucose* and *fructose.* The *three-carbon units* are derivatives of *dihydroxyacetone, glyceraldehyde, glycerate,* and *pyruvate.*

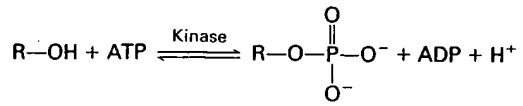




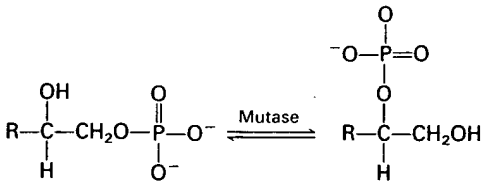
All intermediates between glucose and pyruvate are *phosphorylated.* The phosphoryl groups in these compounds are linked as either *esters* or *anhydrides.*

Now let us look at some of the kinds of reactions that occur in glycolysis and in many other metabolic pathways:

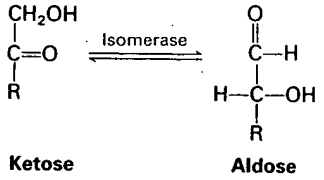
1. *Phosphoryl transfer.* A phosphoryl group is transferred from ATP to a glycolytic intermediate, or from the intermediate to ADP, by a *kinase.*



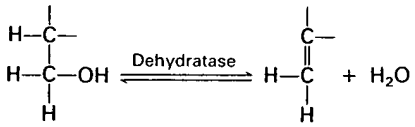
2. *Phosphoryl shift.* A phosphoryl group is shifted from one oxygen atom to another within a molecule by a *mutase.*



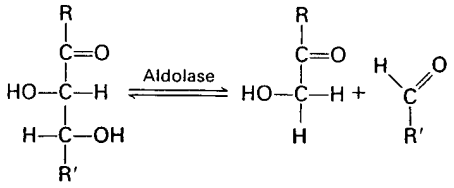
3. *Isomerization.* A ketose is converted into an aldose, or vice versa, by an *isomerase.*



4. *Dehydration.* A molecule of water is eliminated by a *dehydratase.*

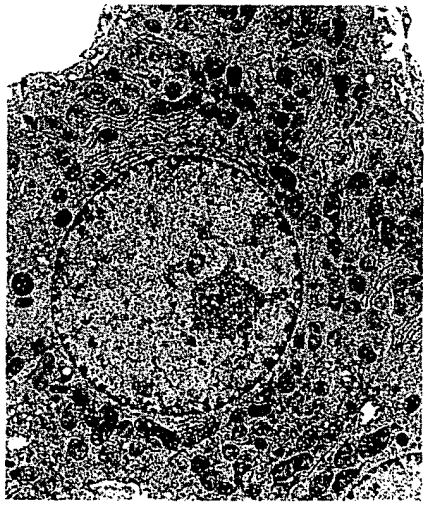


5. *Aldol cleavage.* A carbon–carbon bond is split in a reversal of an aldol condensation by an *aldolase.*



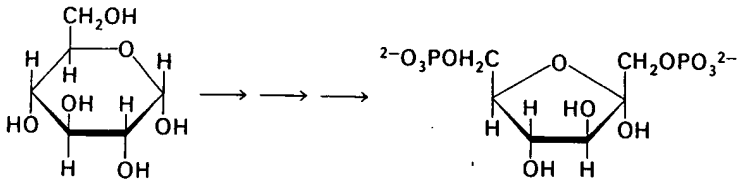
FORMATION OF FRUCTOSE 1,6-BISPHOSPHATE FROM GLUCOSE

We now start our journey down the glycolytic pathway. The reactions in this pathway take place in the cell cytosol. The first stage, which is the conversion of glucose into fructose 1,6-bisphosphate, consists of three steps: a phosphorylation, an isomerization, and a second phosphorylation reaction. *The strategy of these initial steps in glycolysis is to trap the substrate*

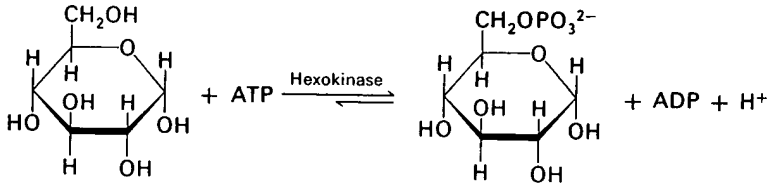


Electron micrograph of a liver cell. Glycolysis takes place in the cytosol. [Courtesy of Dr. Anne Hubbard.]

in the cell and form a compound that can be readily cleaved into phosphorylated three-carbon units. ATP is subsequently extracted from the three-carbon units.



Glucose enters most cells through specific transport proteins and has one principal fate: *it is phosphorylated by ATP to form glucose 6-phosphate.* The transfer of the phosphoryl group from ATP to the hydroxyl group on C-6 of glucose is catalyzed by *hexokinase.*



Phosphoryl transfer is a basic reaction in biochemistry. As was discussed earlier (p. 485), *kinases* are enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor. Hexokinase, then, catalyzes the transfer of a phosphoryl group from ATP to a variety of six-carbon sugars (*hexoses*), such as glucose and mannose. *Hexokinase, like all other kinases, requires Mg^{2+} (or another divalent metal ion such as Mn^{2+}) for activity.* The divalent metal ion forms a complex with ATP. The structures of two possible Mg^{2+}–ATP complexes are shown in Figure 19-2.

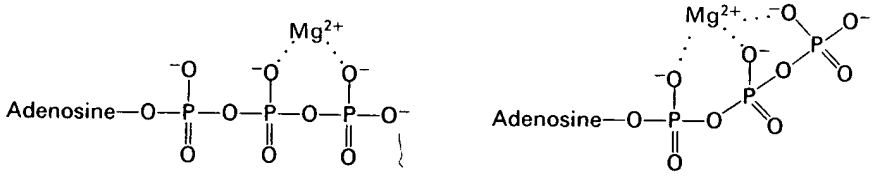
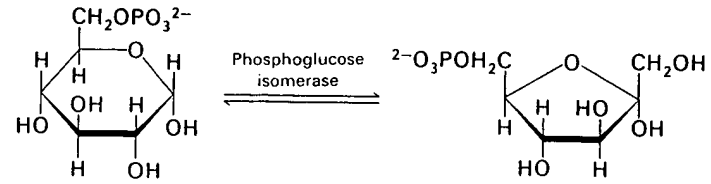
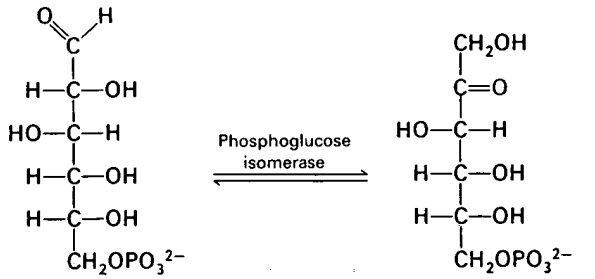


Figure 19-2
Modes of binding Mg^{2+} to ATP.

The next step in glycolysis is the *isomerization of glucose 6-phosphate to fructose 6-phosphate.* The *six-membered pyranose ring* of glucose 6-phosphate is converted into the *five-membered furanose ring* of fructose 6-phosphate. Recall that the open-chain form of glucose has an aldehyde group on C-1, whereas the open-chain form of fructose has a keto group on C-2. The aldehyde on C-1 reacts with the hydroxyl group on C-5 to form the pyranose ring, whereas the keto group on C-2 reacts with the C-5 hydroxyl to form the furanose ring. Thus, the isomerization of glucose 6-phosphate to fructose 6-phosphate is a *conversion of an aldose into a ketose.*

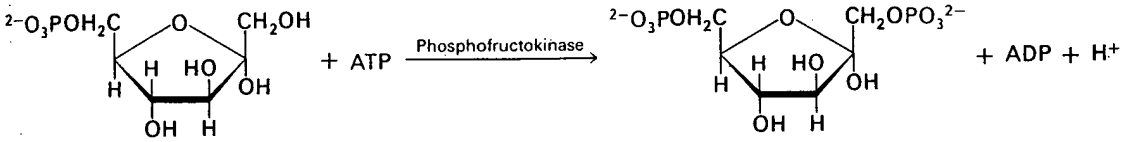


The open-chain representations of these sugars show the essence of this reaction.



Glucose 6-phosphate (An aldose) ⇌ **Fructose 6-phosphate** (A ketose)

A second phosphorylation reaction follows the isomerization step. *Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate.* This compound was formerly known as *fructose 1,6-diphosphate. Bis*phosphate means two separate phosphate groups, whereas *di*phosphate (as in adenosine diphosphate) means two phosphate groups joined by an anhydride bond. Hence, the name *fructose 1,6-bisphosphate* should be used.

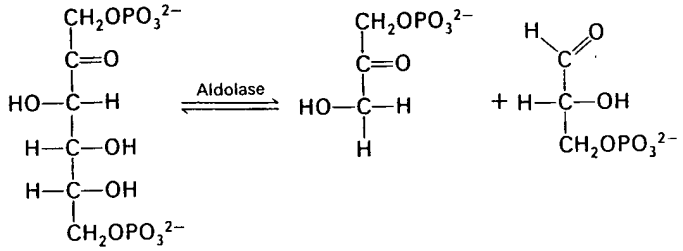


Fructose 6-phosphate → **Fructose 1,6-bisphosphate**

This reaction is catalyzed by *phosphofructokinase,* an allosteric enzyme. The pace of glycolysis is critically dependent on the level of activity of this enzyme, which is allosterically controlled by ATP and several other metabolites (p. 493).

FORMATION OF GLYCERALDEHYDE 3-PHOSPHATE BY CLEAVAGE AND ISOMERIZATION

The second stage of glycolysis consists of four steps, starting with the splitting of fructose 1,6-bisphosphate into *glyceraldehyde 3-phosphate* and *dihydroxyacetone phosphate.* The remaining steps in glycolysis involve three-carbon units rather than six-carbon units.

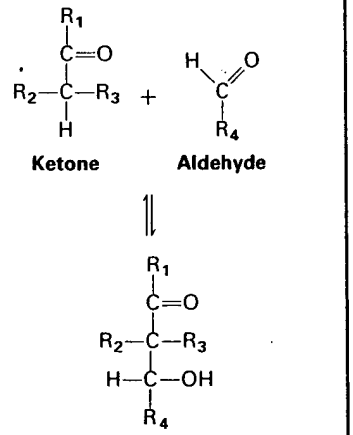


Fructose 1,6-bisphosphate ⇌ **Dihydroxyacetone phosphate** + **Glyceraldehyde 3-phosphate**

This reaction is catalyzed by *aldolase.* This enzyme derives its name from the nature of the reverse reaction, an aldol condensation.

Glyceraldehyde 3-phosphate is on the direct pathway of glycolysis. Dihydroxyacetone phosphate is not, but it can be readily converted into glycer-

Aldol condensation—
The combination of two carbonyl compounds (e.g., an aldehyde and a ketone) to form an aldol (a β-hydroxy-carbonyl compound).



Ketone **Aldehyde**

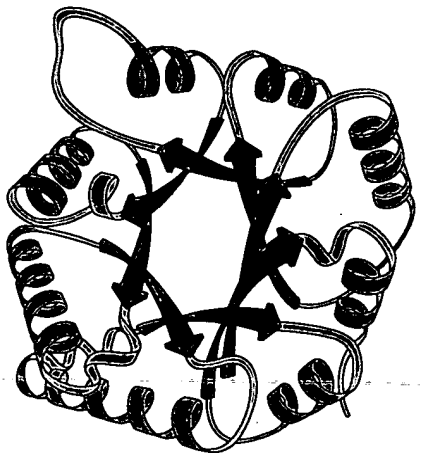
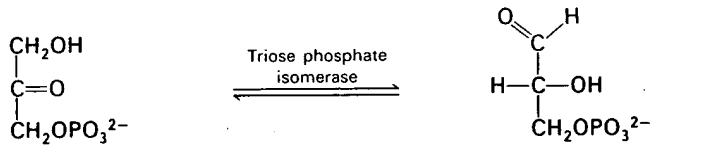


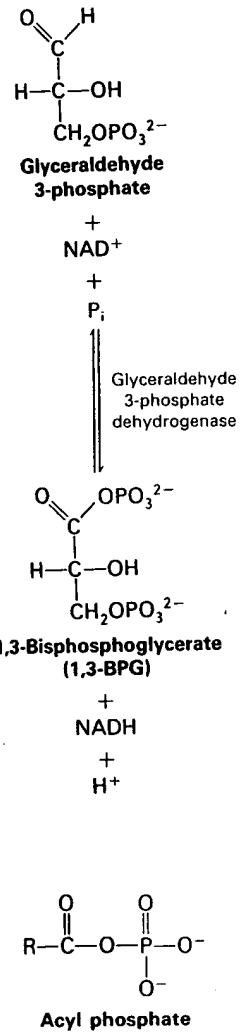
Figure 19-3
Triose phosphate isomerase consists of a central core of eight parallel β strands (red) surrounded by eight α helices (green). Connecting regions are shown in yellow. This structural motif, called an $\alpha\beta$ barrel, is also found in one of the domains of pyruvate kinase. [After a drawing kindly provided by Dr. Jane Richardson.]

aldehyde 3-phosphate. These compounds are isomers: dihydroxyacetone phosphate is a ketose, whereas glyceraldehyde 3-phosphate is an aldose. The isomerization of these three-carbon phosphorylated sugars is catalyzed by *triose phosphate isomerase* (Figure 19-3). This reaction is rapid and reversible. At equilibrium, 96% of the triose phosphate is dihydroxyacetone phosphate. However, the reaction proceeds readily from dihydroxyacetone phosphate to glyceraldehyde 3-phosphate because of efficient removal of this product by subsequent reactions.



Dihydroxyacetone phosphate (A ketose) ⇌ **Glyceraldehyde 3-phosphate** (An aldose)

Thus, two molecules of glyceraldehyde 3-phosphate are formed from one molecule of fructose 1,6-bisphosphate by the sequential action of aldolase and triose phosphate isomerase. The economy of metabolism is evident in this reaction sequence. The isomerase funnels dihydroxyacetone into the main glycolytic pathway—a separate set of reactions are not needed.



ENERGY CONSERVATION: PHOSPHORYLATION IS COUPLED TO THE OXIDATION OF GLYCERALDEHYDE 3-PHOSPHATE

The preceding steps in glycolysis have transformed one molecule of glucose into two molecules of glyceraldehyde 3-phosphate. No energy has yet been extracted. On the contrary, two molecules of ATP have been invested thus far. We come now to a series of steps that harvest some of the energy contained in glyceraldehyde 3-phosphate. The initial reaction in this sequence is the *conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG),* a reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase.* In the earlier literature, 1,3-BPG was known as 1,3-diphosphoglycerate (1,3-DPG).

A *high-potential phosphorylated compound* is generated in this oxidation-reduction reaction. The aldehyde group at C-1 is converted into an *acyl phosphate,* which is a *mixed anhydride* of phosphoric acid and a carboxylic

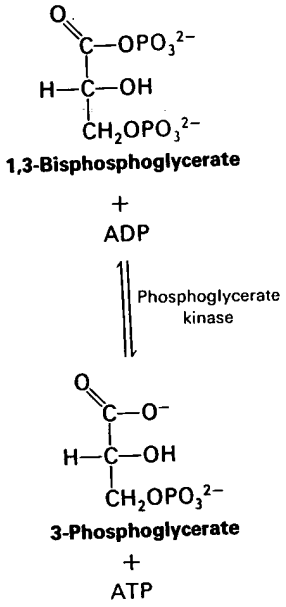
acid. The energy for the formation of this anhydride, which has a high phosphoryl group–transfer potential, comes from the oxidation of the aldehyde group. Note that C-1 in 1,3-BPG is at the oxidation level of a carboxylic acid. NAD^+ (p. 450) is the electron acceptor in this oxidation. The mechanism of this complex reaction, which couples oxidation and phosphorylation, will be discussed later in this chapter (p. 501).

FORMATION OF ATP FROM 1,3-BISPHOSPHOGLYCERATE

In the next step, the high phosphoryl transfer potential of 1,3-BPG is used to generate ATP. Indeed, this is the first ATP-generating reaction in glycolysis. *Phosphoglycerate kinase* catalyzes the transfer of the phosphoryl group from the acyl phosphate of 1,3-BPG to ADP. ATP and 3-phosphoglycerate are the products.

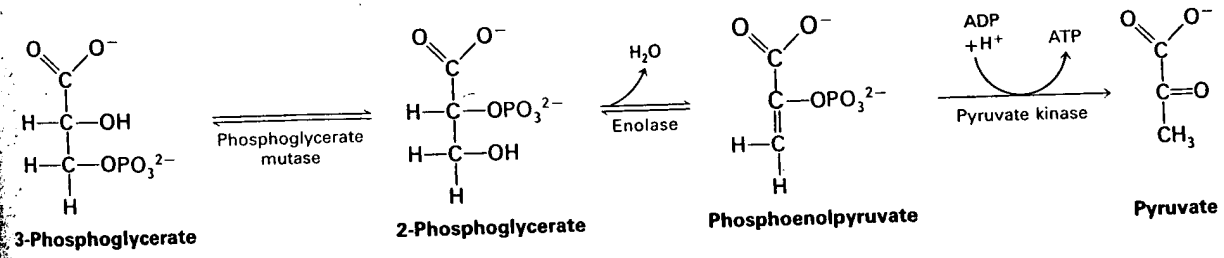
Thus, the outcomes of the reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase are

1. Glyceraldehyde 3-phosphate, an aldehyde, is oxidized to 3-phosphoglycerate, a carboxylic acid.
2. NAD^+ is concomitantly reduced to NADH.
3. ATP is formed from P_i and ADP.



FORMATION OF PYRUVATE AND THE GENERATION OF A SECOND ATP

In the last stage of glycolysis, 3-phosphoglycerate is converted into pyruvate, and a second molecule of ATP is formed.



The first reaction is a rearrangement. The position of the phosphoryl group shifts in the conversion of *3-phosphoglycerate into 2-phosphoglycerate,* a reaction catalyzed by *phosphoglycerate mutase.* In general, a *mutase* is an enzyme that catalyzes the intramolecular shift of a chemical group, such as a phosphoryl group.

In the second reaction, an *enol* is formed by the dehydration of 2-phosphoglycerate. *Enolase* catalyzes the formation of *phosphoenolpyruvate.* This dehydration reaction markedly elevates the group transfer potential of the phosphoryl group. An *enol phosphate* has a high phosphoryl transfer potential, whereas the phosphate ester of an ordinary alcohol has a low one. The reasons for this difference will be discussed later (p. 504).

In the last reaction, *pyruvate* is formed, and ATP is generated concomitantly. The virtually irreversible transfer of a phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by *pyruvate kinase.*

ENERGY YIELD IN THE CONVERSION OF GLUCOSE INTO PYRUVATE

The net reaction in the transformation of glucose into pyruvate is

$$\text{Glucose} + 2\,P_i + 2\,ADP + 2\,NAD^+ \longrightarrow 2\text{ pyruvate} + 2\,ATP + 2\,NADH + 2\,H^+ + 2\,H_2O$$

Thus, *two molecules of ATP are generated in the conversion of glucose into two molecules of pyruvate.* A summary of the steps in which ATP is consumed or formed is given in Table 19-1. Recall that a pair of three-carbon units are formed from fructose 1,6-bisphosphate. The reactions of glycolysis are summarized in Figure 19-4 and Table 19-2.

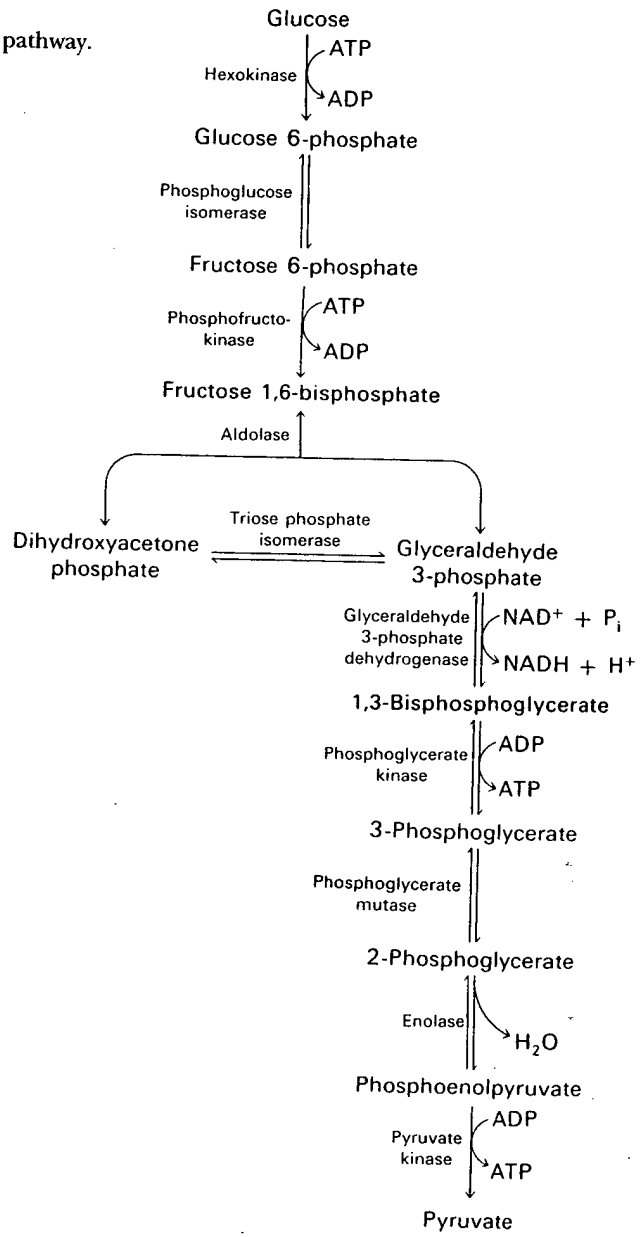


Figure 19-4
The glycolytic pathway.

Table 19-1
Consumption and generation of ATP in glycolysis

Reaction	*ATP change per glucose*
Glucose ⟶ glucose 6-phosphate	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	+2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	+2
Net	+2

Table 19-2
Reactions of glycolysis

Step	*Reaction*	*Enzyme*	*Type**	$\Delta G^{\circ\prime}$	ΔG
1	Glucose + ATP ⟶ glucose 6-phosphate + ADP + H^+	Hexokinase	a	−4.0	−8.0
2	Glucose 6-phosphate ⇌ fructose 6-phosphate	Phosphoglucose isomerase	c	+0.4	−0.6
3	Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	a	−3.4	−5.3
4	Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	e	+5.7	−0.3
5	Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	Triose phosphate isomerase	c	+1.8	+0.6
6	Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	f	+1.5	−0.4
7	1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	a	−4.5	+0.3
8	3-Phosphoglycerate ⇌ 2-phosphoglycerate	Phosphoglycerate mutase	b	+1.1	+0.2
9	2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	Enolase	d	+0.4	−0.8
10	Phosphoenolpyruvate + ADP + H^+ ⟶ pyruvate + ATP	Pyruvate kinase	a	−7.5	−4.0

*Reaction type: (a) phosphoryl transfer; (b) phosphoryl shift; (c) isomerization; (d) dehydration; (e) aldol cleavage; (f) phosphorylation coupled to oxidation.

Note: $\Delta G^{\circ\prime}$ and ΔG are expressed in kcal/mol. ΔG, the actual free-energy change, has been calculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic conditions. Glycolysis can proceed only if the ΔG values of all reactions are negative. The small positive ΔG values of three of the above reactions indicate that the concentrations of metabolites in vivo in cells undergoing glycolysis are not precisely known.

ENTRY OF FRUCTOSE AND GALACTOSE INTO GLYCOLYSIS

Let us consider how two other abundant sugars—fructose and galactose—can be funneled into the glycolytic pathway. Recall that the hydrolysis of sucrose (common table sugar) yields fructose and glucose, and that hydrolysis of lactose (milk sugar) gives galactose and glucose. Fructose itself is present in many foods (e.g., honey). A typical daily intake of fructose is 100 grams. Much of it is metabolized by the liver, using the *fructose 1-phosphate pathway* (Figure 19-5). The first step is the phosphorylation of fructose to fructose 1-phosphate by fructokinase. Fructose 1-phosphate is then split into glyceraldehyde and dihydroxyacetone phosphate. This aldol cleavage is catalyzed by a specific fructose 1-phosphate aldolase. Glyceraldehyde is then phosphorylated to glyceraldehyde 3-phosphate by triose kinase so that it too can enter glycolysis.

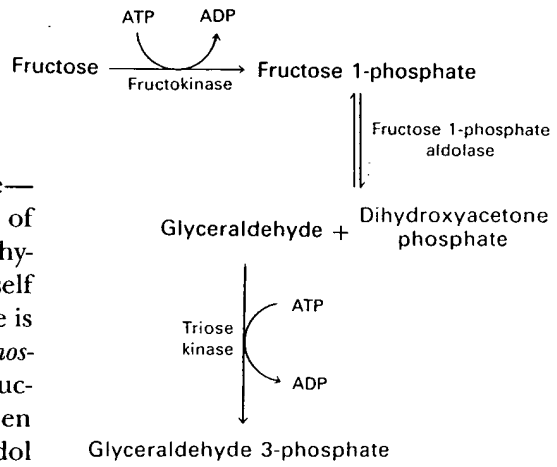


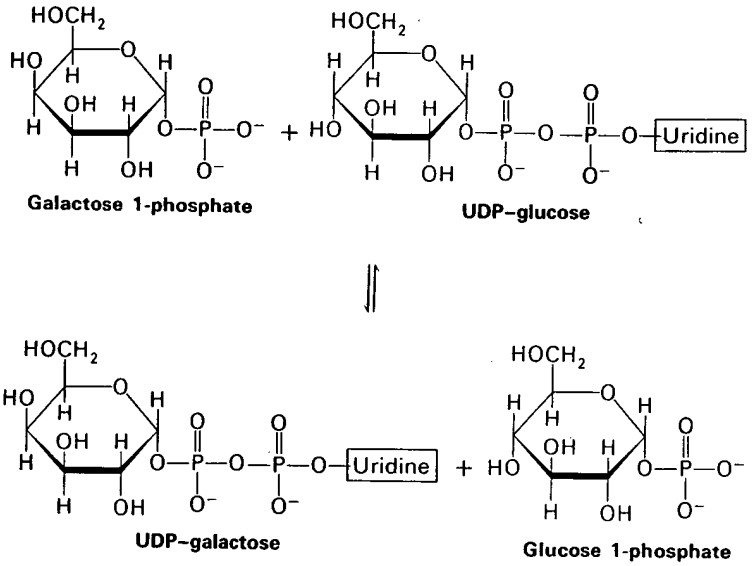
Figure 19-5
Fructose enters the glycolytic pathway via the fructose 1-phosphate pathway.

Alternatively, *fructose can be phosphorylated to fructose 6-phosphate by hexokinase.* However, the affinity of hexokinase for glucose is 20 times as high as it is for fructose. Little fructose 6-phosphate is formed in the liver because of the abundance of glucose relative to fructose in this organ. In contrast, adipose tissue has much more fructose than glucose. Hence, the formation of fructose 6-phosphate is not competitively inhibited to an appreciable extent, and most of the fructose in adipose tissue is metabolized through fructose 6-phosphate.

Galactose is converted into glucose 6-phosphate in four steps. The first reaction in the *galactose-glucose interconversion pathway* is the phosphorylation of galactose to galactose 1-phosphate by *galactokinase.*

$$\text{Galactose} + \text{ATP} \longrightarrow \text{galactose 1-phosphate} + \text{ADP} + \text{H}^+$$

Galactose 1-phosphate then acquires a uridyl group from uridine diphosphate glucose (UDP–glucose), an intermediate in the synthesis of glycosidic linkages (p. 586). The products of this reaction, which is catalyzed by *galactose 1-phosphate uridyl transferase,* are UDP–galactose and glucose 1-phosphate.



The galactose moiety of UDP is then epimerized to glucose. The configuration of the hydroxyl group at C-4 is inverted by *UDP–galactose 4-epimerase.* The sum of the reactions catalyzed by galactokinase, the transferase, and the epimerase is

$$\text{Galactose} + \text{ATP} \longrightarrow \text{glucose 1-phosphate} + \text{ADP} + \text{H}^+$$

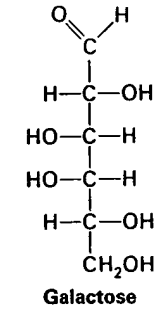
Note that UDP–glucose is not consumed in the conversion of galactose to glucose because it is regenerated from UDP–galactose by the epimerase. This reaction is reversible, and the product of the reverse direction is also important. *The conversion of UDP–glucose into UDP–galactose is essential for the synthesis of galactosyl residues in complex polysaccharides and glycoproteins if the amount of galactose in the diet is inadequate to meet these needs.*

Finally, glucose 1-phosphate, formed from galactose, is isomerized to glucose 6-phosphate by *phosphoglucomutase.* We shall return to this reaction when we consider the synthesis and degradation of glycogen, which proceeds through glucose 1-phosphate (p. 584).

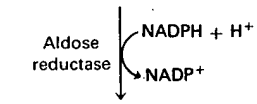
GALACTOSE IS HIGHLY TOXIC IF THE TRANSFERASE IS MISSING

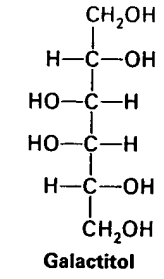
The absence of galactose 1-phosphate uridyl transferase causes *galactosemia,* a severe disease that is inherited as an autosomal recessive trait. The metabolism of galactose in people who have this disease is blocked at galactose 1-phosphate. Afflicted infants fail to thrive. Vomiting or diarrhea occurs when milk is consumed, and enlargement of the liver and jaundice are common. Furthermore, many galactosemics become mentally retarded. The blood galactose level is markedly elevated, and galactose is found in the urine. The absence of the transferase in red blood cells is a definitive diagnostic criterion.

Galactosemia is treated by the exclusion of galactose from the diet. A galactose-free diet leads to a striking regression of virtually all the clinical symptoms, except for mental retardation, which may not be reversible. Continued galactose intake may lead to death in some patients. *The damage in galactosemia is caused by an accumulation of toxic substances, rather than by the absence of an essential compound.* Patients are able to synthesize UDP–galactose from UDP–glucose because their epimerase activity is normal. One of the toxic substances is *galactitol,* which is formed by reduction of galactose. The presence of aldose reductase in the lens of the eye causes galactitol to accumulate there, which leads to the entry of water and the development of cataracts.



Galactose





Galactitol

PHOSPHOFRUCTOKINASE IS THE KEY ENZYME IN THE CONTROL OF GLYCOLYSIS

The glycolytic pathway has a dual role: it degrades glucose to generate ATP, and it provides building blocks for synthetic reactions, such as the formation of long-chain fatty acids. The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs. *In metabolic pathways, enzymes catalyzing essentially irreversible reactions are potential sites of control.* In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible; hence, they would be expected to have regulatory as well as catalytic roles. In fact, each of them serves as a control site. Their activities are regulated by the reversible binding of allosteric effectors or by covalent modification. Also, the amounts of these key enzymes are varied by transcriptional control to meet changing metabolic needs. Reversible allosteric control, regulation by phosphorylation, and transcriptional control typically occur in times of milliseconds, seconds, and hours, respectively.

Phosphofructokinase is the most important control element in the glycolytic pathway of mammals. The enzyme from liver (a 340-kd tetramer) is *inhibited by high levels of ATP,* which lower its affinity for fructose 6-phosphate. A high concentration of ATP converts the hyperbolic binding curve of fructose 6-phosphate into a sigmoidal one (Figure 19-6). This allosteric effect is elicited by the binding of ATP to a specific regulatory site that is distinct from the catalytic site. The inhibitory action of ATP is reversed by AMP, and so *the activity of the enzyme increases when the ATP/AMP ratio is lowered.* In other words, *glycolysis is stimulated as the energy charge falls.* A second control feature comes into play when the pH drops appreciably. The inhibition of phosphofructokinase by H^+ prevents excessive formation of lactate (p. 497) and a precipitous drop in blood pH (acidosis).

Glycolysis also furnishes carbon skeletons for biosyntheses, and so phosphofructokinase should also be regulated by a signal indicating whether

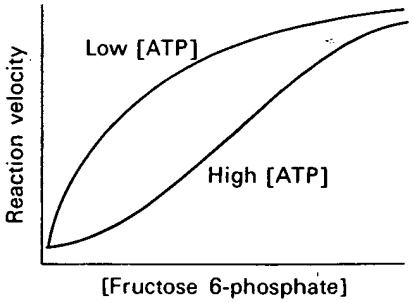
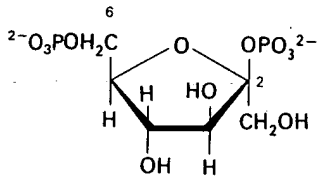


Figure 19-6
Allosteric regulation of phosphofructokinase. A high level of ATP inhibits the enzyme by decreasing its affinity for fructose 6-phosphate. AMP diminishes and citrate enhances the inhibitory effect of ATP.



Fructose 2,6-bisphosphate (F-2,6-BP)

building blocks are abundant or scarce. Indeed, *phosphofructokinase is inhibited by citrate,* an early intermediate in the citric acid cycle (p. 510). A high level of citrate means that biosynthetic precursors are abundant and so additional glucose should not be degraded for this purpose. Citrate inhibits phosphofructokinase by enhancing the inhibitory effect of ATP.

A new regulator of glycolysis was discovered in 1980 by Henri-Géry Hers and Emile Van Schaftingen. They found that *fructose 2,6-bisphosphate,* a previously unknown metabolite, is a potent activator of phosphofructokinase. Fructose 2,6-bisphosphate (F-2,6-BP) activates phosphofructokinase in liver by increasing its affinity for fructose 6-phosphate and diminishing the inhibitory effect of ATP (Figure 19-7). In essence, *F-2,6-BP is an allosteric activator that shifts the conformational equilibrium of this tetrameric enzyme from the T state to the R state.*

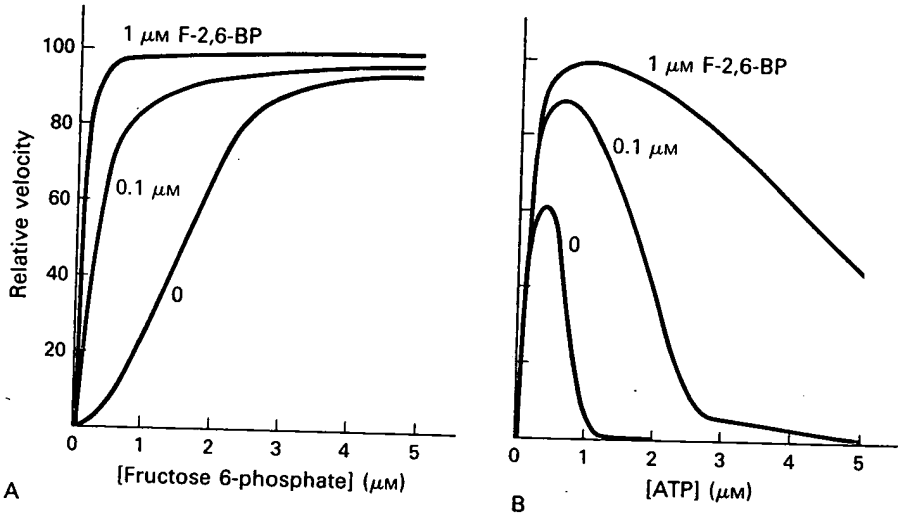
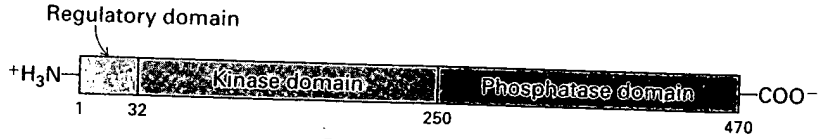


Figure 19-7
Phosphofructokinase is activated by fructose 2,6-bisphosphate. (A) The sigmoidal dependence of velocity on substrate concentration becomes hyperbolic in the presence of 1 μM fructose 2,6-bisphosphate, and (B) the inhibitory effect of ATP is reversed. [After H.-G. Hers and E. Van Schaftingen. *Proc. Nat. Acad. Sci.* 78(1981):2862.]

A REGULATED BIFUNCTIONAL ENZYME SYNTHESIZES AND DEGRADES FRUCTOSE 2,6-BISPHOSPHATE

Fructose 2,6-bisphosphate is formed by the phosphorylation of fructose 6-phosphate, in a reaction catalyzed by phosphofructokinase 2 (PFK2), a different enzyme from phosphofructokinase (PFK) (Figure 19-8). F-2,6-BP is hydrolyzed to fructose 6-phosphate by a specific phosphatase, fructose bisphosphatase 2 (FBPase2). The striking finding is that *both PFK2 and FBPase2 are present in a single 55-kd polypeptide chain.* This bifunctional enzyme contains an N-terminal *regulatory domain,* followed by a *kinase domain* and a *phosphatase domain.* It is interesting to note that PFK2 resem-



Domain structure of bifunctional enzyme

bles phosphofructokinase, whereas FBPase2 resembles phosphoglycerate mutase. The bifunctional enzyme probably arose by the fusion of genes encoding the kinase and phosphatase domains.

Fructose 6-phosphate accelerates the synthesis of F-2,6-BP and inhibits its hydrolysis. Hence, *an abundance of fructose 6-phosphate leads to a higher concentration of F-2,6-BP, which in turn stimulates phosphofructokinase.* Such a process is called *feedforward stimulation.* Furthermore, the activities of

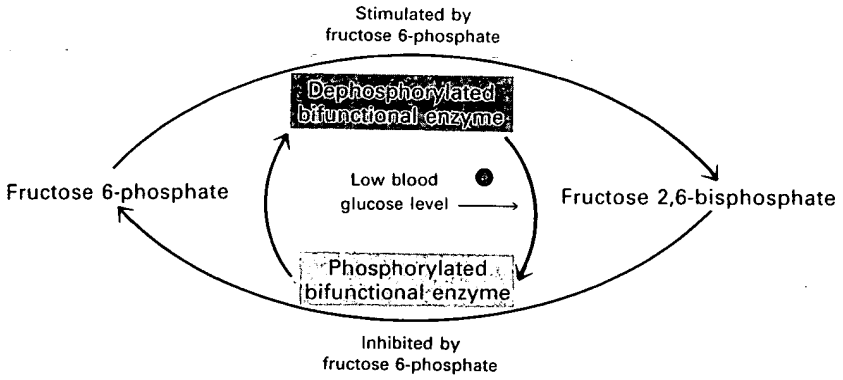


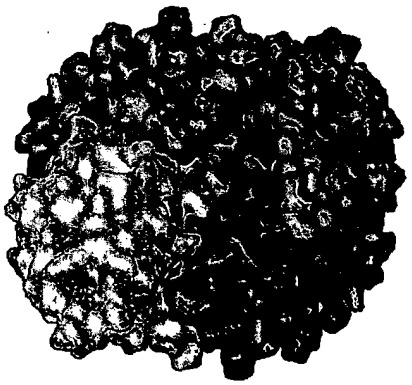
Figure 19-8
Control of the synthesis and degradation of fructose 2,6-bisphosphate. Fructose 6-phosphate accelerates the formation of F-2,6-BP and inhibits its hydrolysis. A low blood glucose level leads to a higher level of the phosphorylated bifunctional enzyme and hence to a lower level of F-2,6-BP.

PFK2 and FBPase2 are reciprocally controlled by *phosphorylation of a single serine residue.* When glucose is scarce, a rise in the blood level of the hormone glucagon triggers a cyclic AMP cascade leading to the phosphorylation of this bifunctional enzyme. This covalent modification activates FBPase2 and inhibits PFK2, lowering the level of F-2,6-BP. Conversely, when glucose is abundant, the enzyme loses its attached phosphate group, which leads to a rise in the level of F-2,6-BP and the consequent acceleration of glycolysis. This coordinated control is facilitated by having the kinase and phosphatase domains on the same polypeptide chain as the regulatory domain. We shall return to this elegant switch when we consider the integration of carbohydrate metabolism (p. 766).

HEXOKINASE AND PYRUVATE KINASE ALSO SET THE PACE OF GLYCOLYSIS

Hexokinase, the enzyme catalyzing the first step of glycolysis, is inhibited by glucose 6-phosphate. When phosphofructokinase is inactive, the concentration of fructose 6-phosphate rises. In turn, the level of glucose 6-phosphate rises because it is in equilibrium with fructose 6-phosphate. Hence, *the inhibition of phosphofructokinase leads to the inhibition of hexokinase.* However, liver possesses *glucokinase,* a specialized isoform of hexokinase that is not inhibited by glucose 6-phosphate. Glucokinase phosphorylates glucose only when it is abundant because it has a much higher K_M for glucose than does hexokinase (5 mM, compared with 0.1 mM). The role of glucokinase is to provide glucose 6-phosphate for the synthesis of glycogen, a storage form of glucose (p. 586). The high K_M of glucokinase in the liver gives brain and muscle first call on glucose when its supply is limited.

Why is phosphofructokinase rather than hexokinase the pacemaker of glycolysis? The reason becomes evident on noting that glucose 6-phosphate is not solely a glycolytic intermediate. Glucose 6-phosphate can also be converted into glycogen or it can be oxidized by the pentose phosphate pathway (p. 559) to form NADPH. The first irreversible reaction



Structure of a bacterial phosphofructokinase. The four identical subunits are shown in different colors in a model depicting the surface of the enzyme. [Courtesy of Dr. Anthony Nicholls. Drawn from 4pfk.pdb. T. Schirmer and P.R. Evans. *Nature* 343(1990):140.]

unique to the glycolytic pathway, called the *committed step,* is the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Thus, it is highly appropriate for phosphofructokinase to be the primary control site in glycolysis. In general, *the enzyme catalyzing the committed step in a metabolic sequence is the most important control element in the pathway.*

Pyruvate kinase, the enzyme catalyzing the third irreversible step in glycolysis, controls the outflow from this pathway. This final step yields ATP and pyruvate, a central metabolic intermediate that can be oxidized further or used as a building block. Several forms of pyruvate kinase (a tetramer of 57-kd subunits) encoded by different genes are present in mammals: the L type predominates in liver, and the M type in muscle and brain. These variations on a common theme, called *isoenzymes* or *isozymes,* have essentially the same architectural plan and catalytic mechanism but differ in how they are regulated. Both the L and M forms bind phosphoenolpyruvate cooperatively. Fructose 1,6-bisphosphate, the product of the preceding irreversible step in glycolysis, activates pyruvate kinase to enable it to keep pace with the oncoming high flux of intermediates. ATP allosterically inhibits pyruvate kinase to slow glycolysis when the energy charge is high (Figure 19-9).

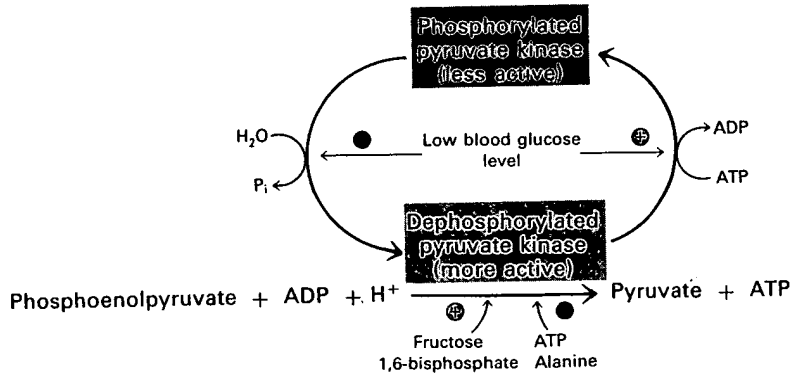


Figure 19-9
Control of the catalytic activity of pyruvate kinase.

Alanine (synthesized in one step from pyruvate, p. 630) also allosterically inhibits pyruvate kinase, in this case to signal that building blocks are abundant. The catalytic properties of the L form—but not of the M form—are also controlled by reversible phosphorylation. When the blood glucose level is low, glucagon triggers a cyclic AMP cascade that leads to the phosphorylation of pyruvate kinase, which diminishes its activity. A rise in the cytosolic calcium level induced by hormones such as vasopressin also leads to phosphorylation and inhibition of pyruvate kinase. *These hormone-triggered phosphorylations, like that of the bifunctional enzyme controlling the levels of fructose 2,6-bisphosphate, prevent the liver from consuming glucose when it is more urgently needed by brain and muscle* (p. 575). We see here a clear-cut example of how isoenzymes contribute to the metabolic diversity of different organs.

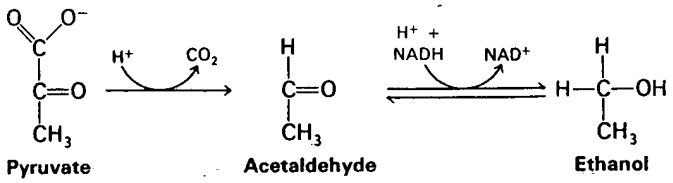
DIVERSE FATES OF PYRUVATE: ETHANOL, LACTATE, OR ACETYL COENZYME A

The sequence of reactions from glucose to pyruvate is similar in all organisms and in all kinds of cells. In contrast, the fate of pyruvate is variable. Three reactions of pyruvate are of prime importance:

1. *Ethanol* is formed from pyruvate in yeast and several other microorganisms. The first step is the decarboxylation of pyruvate.

$$\text{Pyruvate} + H^+ \longrightarrow \text{acetaldehyde} + CO_2$$

This reaction is catalyzed by *pyruvate decarboxylase.* Thiamine pyrophosphate, the coenzyme here, also participates in reactions catalyzed by decarboxylases (p. 516) and other enzymes (p. 566).



The second step is the reduction of acetaldehyde to ethanol by NADH, in a reaction catalyzed by *alcohol dehydrogenase.*

$$\text{Acetaldehyde} + \text{NADH} + H^+ \rightleftharpoons \text{ethanol} + NAD^+$$

The active site of alcohol dehydrogenase contains a zinc ion that is coordinated to the sulfur atoms of two cysteine residues and a histidine nitrogen atom (Figure 19-10). As in carboxypeptidase A (p. 221), Zn^{2+} polarizes the carbonyl group of the substrate to stabilize the transition state (Figure 19-11).

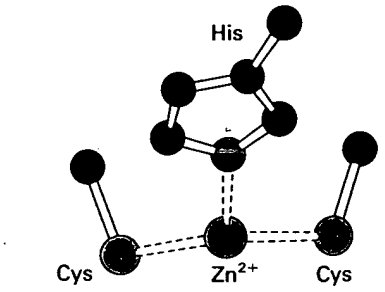


Figure 19-10
Model of the active site of liver alcohol dehydrogenase showing the coordination of the zinc ion. [Drawn from 2ohx.pdb; coordinates deposited by S. Al-Karadaghi and E.S. Cedergren-Zeppezauer.]

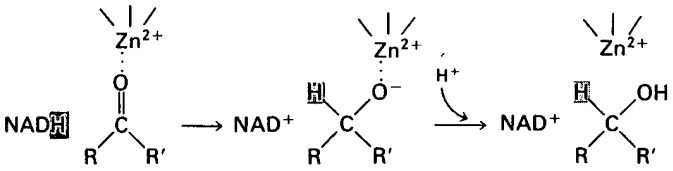


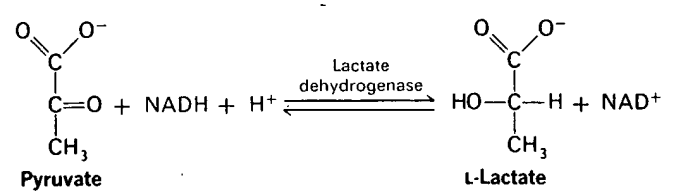
Figure 19-11
Catalytic mechanism of alcohol dehydrogenase.

The conversion of glucose into ethanol is called *alcoholic fermentation.* The net result of this anaerobic process is

$$\text{Glucose} + 2\,P_i + 2\,\text{ADP} + 2\,H^+ \longrightarrow 2\,\text{ethanol} + 2\,CO_2 + 2\,\text{ATP} + 2\,H_2O$$

It is important to note that NAD^+ and NADH do not appear in this equation, even though they are crucial for the overall reaction. NAD^+ generated in the reduction of acetaldehyde to ethanol is consumed in the oxidation of glyceraldehyde 3-phosphate. Thus, *there is no net oxidation-reduction in the conversion of glucose into ethanol.*

2. *Lactate* is normally formed from pyruvate in a variety of microorganisms. The reaction also occurs in the cells of higher organisms when the amount of oxygen is limiting, as in muscle during intense activity. The reduction of pyruvate by NADH to form lactate is catalyzed by *lactate dehydrogenase.*



The overall reaction in the conversion of glucose into lactate is

$$\text{Glucose} + 2\,P_i + 2\,\text{ADP} \longrightarrow 2\,\text{lactate} + 2\,\text{ATP} + 2\,H_2O$$

As in alcoholic fermentation, there is no net oxidation-reduction. The NADH formed in the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of pyruvate. *The regeneration of NAD^+ in the reduction of pyruvate to lactate or ethanol sustains the continued operation of glycolysis under anaerobic conditions.* If NAD^+ were not regenerated, glycolysis could not proceed beyond glyceraldehyde 3-phosphate, which means that no ATP would be generated. In effect, the formation of lactate by aerobic organisms buys time, as will be discussed in Chapter 22.

3. Only a small fraction of the energy of glucose is released in its anaerobic conversion into lactate (or ethanol). Much more energy can be extracted aerobically by means of the citric acid cycle and the electron transport chain. The entry point to this oxidative pathway is *acetyl coenzyme A (acetyl CoA)*, which is formed inside mitochondria by the oxidative decarboxylation of pyruvate.

$$\text{Pyruvate} + NAD^+ + \text{CoA} \longrightarrow \text{acetyl CoA} + CO_2 + NADH$$

This reaction, which is catalyzed by the pyruvate dehydrogenase complex, will be discussed in detail in the next chapter. The NAD^+ required for this reaction and for the oxidation of glyceraldehyde 3-phosphate is regenerated when NADH ultimately transfers its electrons to O_2 through the electron transport chain in mitochondria.

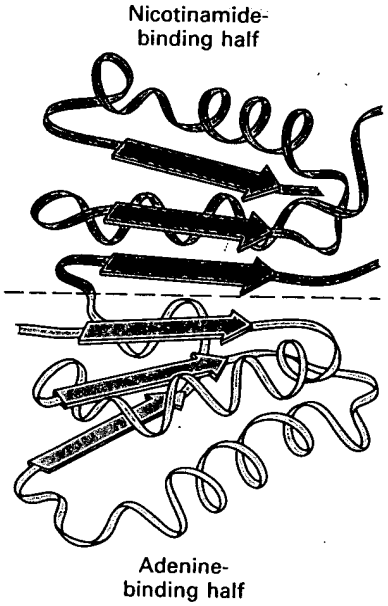


Figure 19-12
Schematic diagram of the NAD^+-binding region in dehydrogenases. The nicotinamide-binding half (shaded green) is structurally similar to the adenine-binding half (shaded yellow) of the site. [After M.G. Rossmann, A. Liljas, C.-I. Brändén, and L.J. Banaszak. In *The Enzymes,* vol. 10, 3rd ed. (Academic Press, 1975), p. 68.]

THE BINDING SITE FOR NAD^+ IS VERY SIMILAR IN MANY DEHYDROGENASES

Lactate dehydrogenase from skeletal muscle, a 140-kd tetramer, and alcohol dehydrogenase, an 84-kd dimer, have quite different three-dimensional structures. However, their NAD^+-binding domains are strikingly similar (Figure 19-12). This nucleotide-binding region is made up of four α helices and a sheet of six parallel β strands. The conformations of NAD^+ bound to lactate dehydrogenase and to alcohol dehydrogenase also are nearly the same. The adenine moiety of NAD^+ is bound in a hydrophobic crevice. In contrast, the nicotinamide unit is bound so that the reactive side of the ring is in a polar environment, whereas the other side makes contact with hydrophobic residues of the enzyme. The bound NAD^+ has an extended conformation (Figure 19-13). The three-dimen-

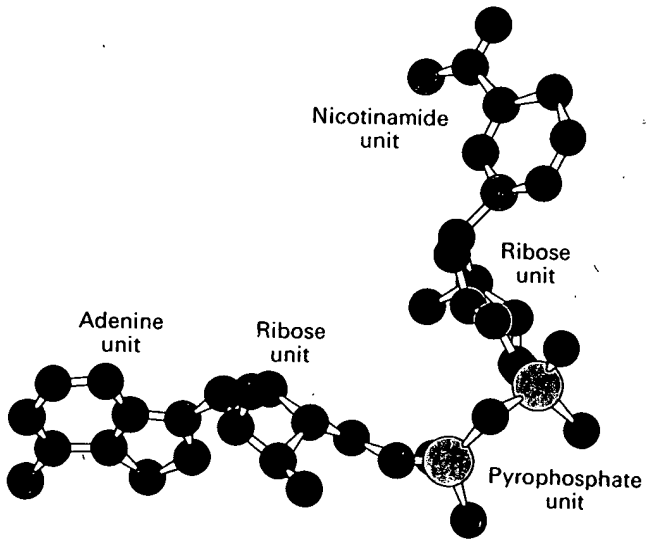


Figure 19-13
Model of NAD^+. The conformation shown here is the one found in the complex of NAD^+ and lactate dehydrogenase.

sional structures of two other NAD^+-requiring enzymes—glyceraldehyde 3-phosphate dehydrogenase and malate dehydrogenase (an enzyme of the citric acid cycle, p. 512)—are also known at high resolution. Their NAD^+-binding domains closely resemble those of lactate dehydrogenase and alcohol dehydrogenase. *The NAD^+-binding region common to these four enzymes is a fundamental structural motif of NAD^+-linked dehydrogenases.*

INDUCED FIT IN HEXOKINASE: GLUCOSE CLOSES THE ACTIVE-SITE CLEFT

X-ray crystallographic studies of yeast hexokinase have revealed that the binding of glucose leads to a large conformational change in the enzyme. Hexokinase consists of two lobes, which come together when glucose is bound (Figure 19-14). Glucose induces a 12-degree rotation of one lobe with respect to the other, resulting in movements of the polypeptide backbone of as much as 8 Å. The cleft between the lobes closes, and the bound glucose becomes surrounded by protein, except for its 5-hydroxymethyl group.

The closing of the cleft in hexokinase is a striking example of the role of *induced fit* in enzyme action, as originally proposed by Koshland (p. 191). The glucose-induced structural changes are likely to be significant in two ways. First, the environment around the glucose becomes much more nonpolar, which encourages the donation of the terminal phosphoryl group of ATP. Second, the embracing of glucose by hexokinase enables the enzyme to discriminate against H_2O as a substrate. If hexokinase were rigid, a water molecule occupying the binding site for the $-CH_2OH$ of glucose would attack the γ-phosphoryl group of ATP. In other words, a rigid kinase would necessarily be an ATPase as well as a kinase. The undesirable ATPase activity is prevented by making hexokinase enzymatically active only when glucose closes the cleft. It is interesting to note that pyruvate kinase, phosphoglycerate kinase, and phosphofructokinase also contain clefts between lobes that close when substrate is bound. *Substrate-induced cleft closing is likely to be a general feature of kinases.*

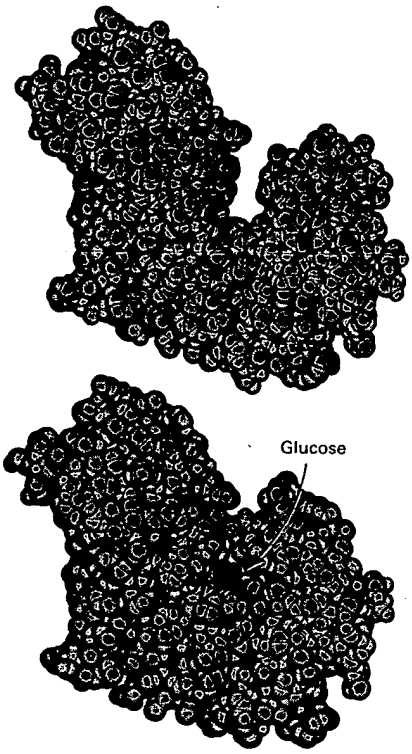
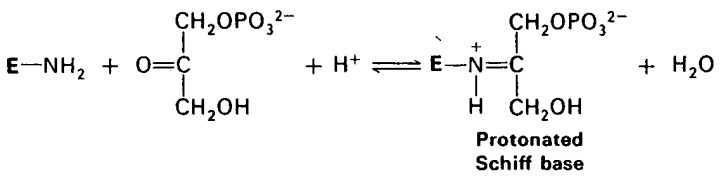


Figure 19-14
The conformation of hexokinase changes markedly on binding glucose (shown in red). The two lobes of the enzyme come together and surround the substrate. [Courtesy of Dr. Thomas Steitz.]

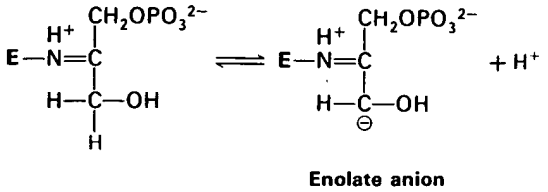
ALDOLASE FORMS A SCHIFF BASE WITH DIHYDROXYACETONE PHOSPHATE

Let us turn now to the catalytic mechanism of aldolase. For convenience, we will examine the condensation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate to form fructose 1,6-bisphosphate, the reverse of the glycolytic reaction. First, dihydroxyacetone phosphate forms a protonated Schiff base with a specific lysine residue in the active site of animal aldolases.

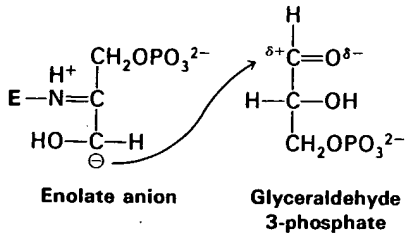


In this reaction, a nucleophile (the amino group) attacks the carbonyl group to form a tetrahedral intermediate, which then dehydrates. *The resulting protonated Schiff base plays a critical role in catalysis. It promotes the*

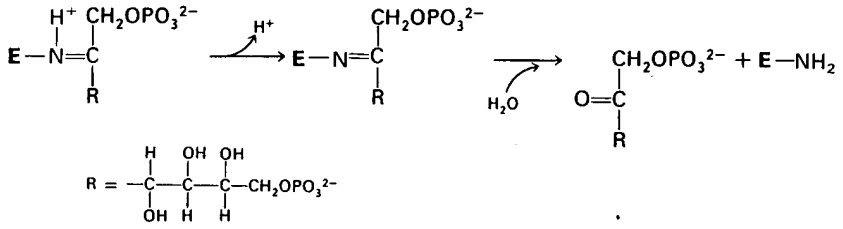
formation of the enolate anion of dihydroxyacetone phosphate by serving as an electron sink (a potent electron acceptor).



The enolate anion then adds to the aldehyde group of glyceraldehyde 3-phosphate to form a protonated Schiff base of fructose 1,6-bisphosphate.



The Schiff base is deprotonated and hydrolyzed to yield fructose 1,6-bisphosphate and the regenerated enzyme.

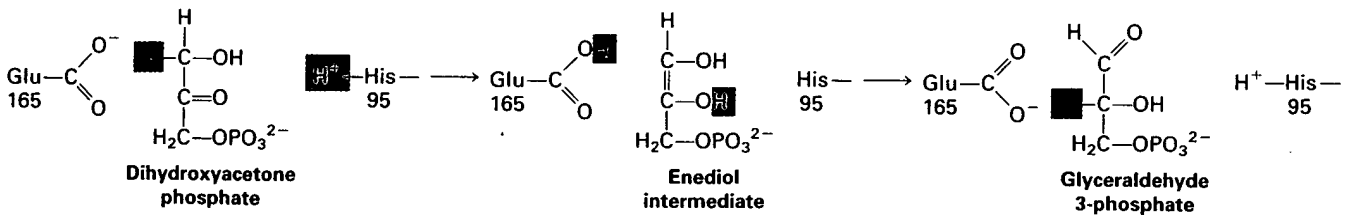


The pathway for the cleavage of fructose 1,6-bisphosphate is simply the reverse of the one for its formation.

KINETIC PERFECTION IN CATALYSIS: TRIOSE PHOSPHATE ISOMERASE IN ACTION

Much is known about the catalytic mechanism of triosephosphate isomerase (TIM), an especially interesting enzyme. TIM catalyzes the transfer of a hydrogen atom from C-1 to C-2 in converting dihydroxyacetone phosphate into glyceraldehyde 3-phosphate, an intramolecular oxidation-reduction. This isomerization of a ketose into an aldose proceeds through an *enediol intermediate* (Figure 19-15). When the reaction is carried out in D_2O, deuterium becomes incorporated into C-2. This finding rules out a

Figure 19-15
Catalytic mechanism of triosephosphate isomerase.



direct transfer of a hydride ion ($:H^-$) from C-1 to C-2. Rather, the first step is the removal of a proton from C-1 by a basic group of the protein to form an enediol. Isotope labeling studies of the reverse reaction provided another key clue. Some of the tritium attached to C-2 of specifically labeled glyceraldehyde 3-phosphate emerged in C-1 of dihydroxyacetone phosphate, whereas the rest exchanged with protons of water. This finding revealed that *abstraction and donation of a proton are mediated by the same catalytic base.*

X-ray crystallographic studies then showed that glutamate 165 plays this role (Figure 19-16). However, a carboxylate group by itself is not basic enough to pull a proton away from a carbon atom adjacent to a carbonyl group. Histidine 95 assists catalysis by donating a proton to the C-2 carbonyl group. The phosphate group of the substrate is held in place by a salt bridge with lysine 12 and hydrogen bonds with two main-chain NH groups. The positively charged end of an α helix dipole is also trained on the dianionic phosphate moiety.

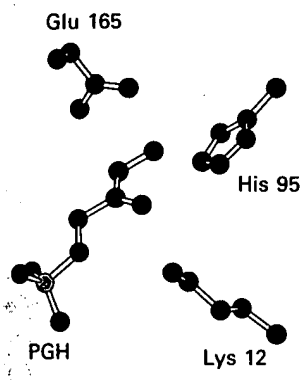


Figure 19-16
Active site of triosephosphate isomerase. Phosphoglycolhydroxamate (PGH), a substrate analog, is tightly bound. Glu 165 and His 95 play key catalytic roles. Lys 12 forms a salt bridge with the phosphate group of the substrate analog and polarizes the C-2 oxygen atom. [Drawn from 7tim.pdb. R.C. Davenport, P.A. Bash, B.A. Seaton, M. Karplus, G.A. Petsko, and D. Ringe. *Biochemistry* 30(1991):5821.]

Two other features of this isomerase are noteworthy. First, TIM displays great catalytic prowess. It accelerates isomerization by a factor of 10^{10} compared with that obtained with a simple base catalyst such as acetate ion. Indeed, the k_{cat}/K_M ratio for isomerization of glyceraldehyde 3-phosphate is $2 \times 10^8\ M^{-1}\ s^{-1}$, which is close to the diffusion-controlled limit. In other words, the rate-limiting step in catalysis is the diffusion-controlled encounter of substrate and enzyme. TIM is an example of a *kinetically perfect enzyme* (p. 195). Second, TIM suppresses an undesired side reaction, the decomposition of the enediol intermediate into methylglyoxal and P_i (Figure 19-17). In solution, this unfavorable reaction is 100 times faster than isomerization. Hence, TIM must prevent the enediol from leaving the enzyme. This labile intermediate is sequestered in the active site by the movement of a loop of 10 residues. This lid shuts the active site when the enediol is present, and reopens when isomerization is completed. *We see here a striking example of the channeling of catalysis by induced fit.*

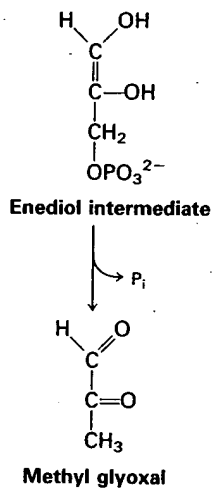


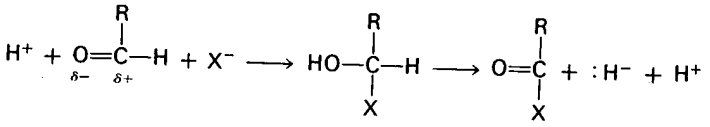
Figure 19-17
In solution, the enediol intermediate in the isomerization of dihydroxyacetone phosphate decomposes into methylglyoxal and P_i. The isomerase blocks this undesired side reaction.

A THIOESTER IS FORMED IN THE OXIDATION OF GLYCERALDEHYDE 3-PHOSPHATE

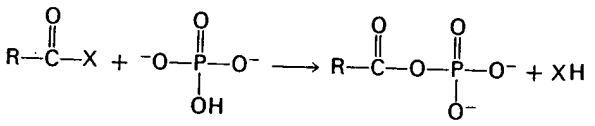
Glyceraldehyde 3-phosphate dehydrogenase catalyzes the oxidative phosphorylation of its aldehyde substrate.

$$\text{Glyceraldehyde 3-phosphate} + P_i + NAD^+ \longrightarrow \text{1,3-BPG} + NADH + H^+$$

The conversion of an aldehyde into an acyl phosphate entails both *oxidation* and *phosphorylation.* Oxidation requires the *removal of a hydride ion* ($:H^-$), which is a hydrogen nucleus and two electrons. Removal of a hydride ion from an aldehyde is energetically costly because of the dipolar character of the carbonyl group: the carbon atom of the carbonyl group already has a partial positive charge. Removal of the hydride ion is greatly facilitated by making the carbon atom less positively charged. This is accomplished by the *addition of a nucleophile,* represented here by X^-:



The hydride ion readily leaves the addition compound because the carbon atom no longer carries a large positive charge. Furthermore, some of the free energy of the oxidation is preserved in the acyl intermediate. Addition of orthophosphate to this acyl intermediate yields an acyl phosphate, which has a high group transfer potential.



Now let us see how glyceraldehyde 3-phosphate dehydrogenase carries out these steps (Figure 19-18). *The nucleophile X^- is the sulfhydryl group of a cysteine residue at the active site.* The aldehyde substrate reacts with the ionized form of this sulfhydryl group to form a hemithioacetal. The next step is the *transfer of a hydride ion to a molecule of NAD^+ that is tightly bound to the enzyme.* The products of this reaction are the reduced coenzyme NADH and a thioester. *This thioester is an energy-rich intermediate,* corresponding to the acyl intermediate mentioned earlier. NADH dissociates from the enzyme, and another NAD^+ binds to the active site. Orthophosphate then attacks the thioester to form 1,3-BPG, a high-potential phosphoryl donor.

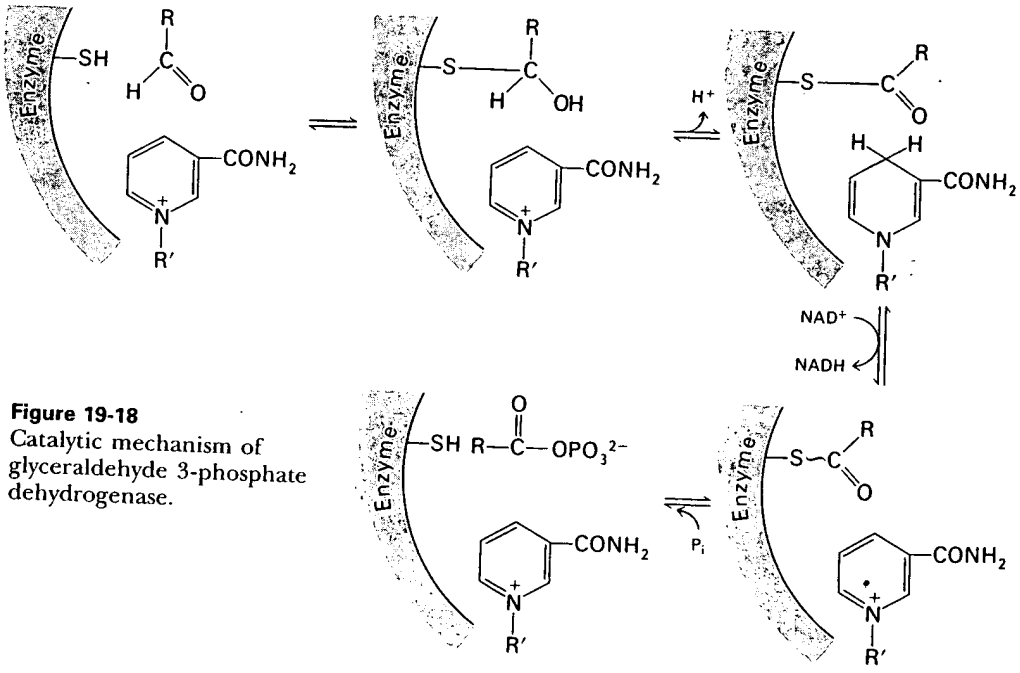
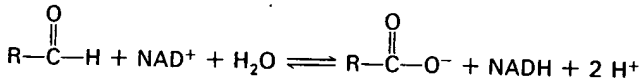


Figure 19-18
Catalytic mechanism of glyceraldehyde 3-phosphate dehydrogenase.

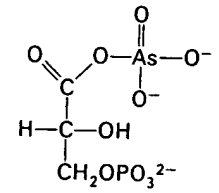
A crucial aspect of the formation of 1,3-BPG from glyceraldehyde 3-phosphate is that a thermodynamically unfavorable reaction, the formation of an acyl phosphate from a carboxylate, is driven by a thermodynamically favorable reaction, the oxidation of an aldehyde.



These two reactions are *coupled by the thioester intermediate,* which preserves much of the free energy released in the oxidation reaction. We see here the *use of a covalent enzyme-bound intermediate as a mechanism of energy coupling.*

ARSENATE, AN ANALOG OF PHOSPHATE, POISONS BY UNCOUPLING OXIDATION AND PHOSPHORYLATION

Arsenate (AsO_4^{3-}) closely resembles P_i in structure and reactivity. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, arsenate can replace phosphate in attacking the energy-rich thioester intermediate. The product of this reaction, 1-arseno-3-phosphoglycerate, is unstable, in contrast with 1,3-bisphosphoglycerate. 1-Arseno-3-phosphoglycerate and other acyl arsenates are rapidly and spontaneously hydrolyzed. Hence, the net reaction in the presence of arsenate is



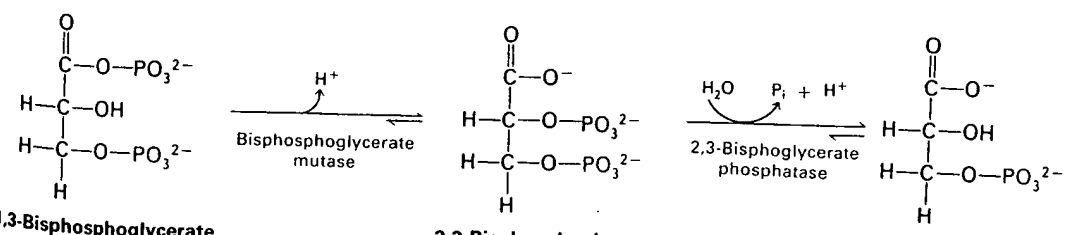
1-Arseno-3-phosphoglycerate

Glyceraldehyde 3-phosphate + NAD^+ + H_2O ⟶
3-phosphoglycerate + NADH + 2 H^+

Note that glycolysis proceeds in the presence of arsenate but that the ATP normally formed in the conversion of 1,3-bisphosphoglycerate into 3-phosphoglycerate is lost. Thus, *arsenate uncouples oxidation and phosphorylation by forming a highly labile acyl arsenate.* Arsenic is a potent poison because arsenate generally substitutes for phosphate in phosphoryl transfer reactions. Also, arsenite (AsO_2^-) forms adducts with thiols. One likely reason for the choice of phosphorus over arsenic in the evolution of biomolecules is the greater kinetic stability of its energy-rich compounds.

2,3-BISPHOSPHOGLYCERATE, AN ALLOSTERIC EFFECTOR OF HEMOGLOBIN, ARISES FROM 1,3-BISPHOSPHOGLYCERATE

We have seen that fructose 2,6-bisphosphate, a regulatory molecule, arises from a glycolytic intermediate. Another regulatory molecule coming from this pathway is *2,3-bisphosphoglycerate* (2,3-BPG), a controller of oxygen transport in erythrocytes (p. 160). Red blood cells have a high concentration of 2,3-BPG, typically 4 mM, in contrast with most other cells, which have only trace amounts. The synthesis and degradation of 2,3-BPG are a short detour from the glycolytic pathway (Figure 19-19).



1,3-Bisphosphoglycerate (1,3-BPG) — **2,3-Bisphosphoglycerate (2,3-BPG)** — **3-Phosphoglycerate**

Figure 19-19
Synthesis and degradation of 2,3-bisphosphoglycerate.

Table 19-3
Typical concentrations of glycolytic intermediates in erythrocytes

Intermediate	*μM*
Glucose	5000
Glucose 6-phosphate	83
Fructose 6-phosphate	14
Fructose 1,6-bisphosphate	31
Dihydroxyacetone phosphate	138
Glyceraldehyde 3-phosphate	19
1,3-Bisphosphoglycerate	1
2,3-Bisphosphoglycerate	4000
3-Phosphoglycerate	118
2-Phosphoglycerate	30
Phosphoenolpyruvate	23
Pyruvate	51
Lactate	2900
ATP	1850
ADP	138
P_i	1000

After S. Minakami and H. Yoshikawa. *Biochem. Biophys. Res. Comm.* 18(1965):345.

Bisphosphoglycerate mutase converts 1,3-BPG into 2,3-BPG. 2,3-BPG is a potent competitive inhibitor of its own formation. The concentration of this allosteric effector also depends on its hydrolysis to 3-phosphoglycerate, a reaction catalyzed by *2,3-bisphosphoglycerate phosphatase.* Both the synthesis and degradation of 2,3-BPG are nearly irreversible.

The mutase reaction has an interesting mechanism. *3-Phosphoglycerate* is an obligatory participant although it does not appear in the overall stoichiometry. The mutase binds 1,3-BPG and 3-phosphoglycerate simultaneously. In this ternary complex, a phosphoryl group is transferred from position 1 of 1,3-BPG to position 2 of 3-phosphoglycerate (Figure 19-20).

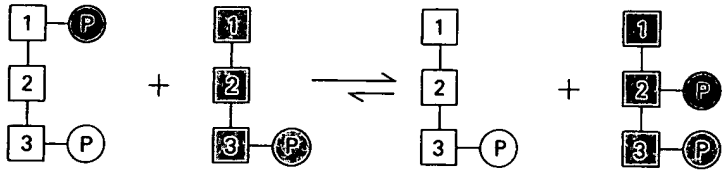
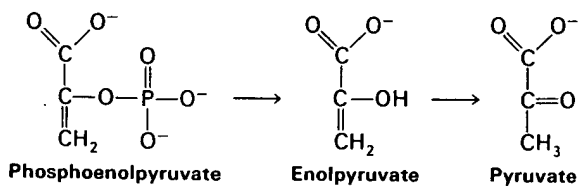


Figure 19-20
3-Phosphoglycerate participates in the conversion of 1,3-bisphosphoglycerate into 2,3-bisphosphoglycerate.

2,3-BPG is important not only in red blood cells. Phosphoglycerate mutase, the enzyme catalyzing the interconversion of 3-phosphoglycerate and 2-phosphoglycerate, requires a catalytic amount of 2,3-BPG to be active. 2,3-BPG phosphorylates a histidine residue at the catalytic site to maintain the active form of the enzyme. Thus, 2,3-bisphosphoglycerate plays an essential role in basic metabolism. It seems likely that 2,3-BPG was present eons before it was recruited by red cells to control the oxygen affinity of hemoglobin.

ENOL PHOSPHATES ARE POTENT PHOSPHORYL DONORS

Because it is an *acyl phosphate,* 1,3-BPG has a high group transfer potential. A different kind of high-energy phosphate compound is generated several steps later in glycolysis. Phosphoenolpyruvate, an *enol phosphate,* is formed by the dehydration of 2-phosphoglycerate. The $\Delta G^{\circ\prime}$ of hydrolysis of a phosphate ester of an ordinary alcohol is -3 kcal/mol, whereas that of phosphoenolpyruvate is -14.8 kcal/mol. Why does phosphoenolpyruvate have such a high phosphoryl group–transfer potential? The answer is that the enol formed upon transfer of the phosphoryl group undergoes a conversion into a ketone—namely, pyruvate.



The $\Delta G^{\circ\prime}$ of the enol-ketone conversion is very large, of the order of -10 kcal/mol. Thus, *the high phosphoryl group–transfer potential of phosphoenolpyruvate arises primarily from the large driving force of the subsequent enol-ketone conversion.*

A FAMILY OF TRANSPORTERS ENABLES GLUCOSE TO ENTER AND LEAVE ANIMAL CELLS

The thermodynamically downhill movement of glucose across the plasma membrane of animal cells is mediated by several *glucose transporters*. The members of this protein family, named GLUT1 to 5, consist of a single polypeptide chain about 500 residues long. The common structural theme is the presence of 12 transmembrane segments (Figure 19-21). The binding site for glucose alternately faces the inside and outside of the cell when occupied by a sugar; this eversion is accomplished by conformational changes within the transporter and not by a rotation of the whole protein.

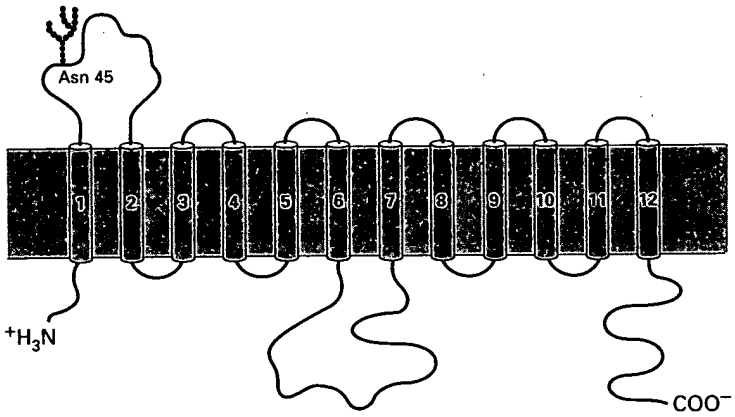


Figure 19-21
Model of a mammalian glucose transporter. The hydrophobicity profile of the protein points to the presence of 12 transmembrane α helices. Chemical labeling and tryptic cleavage studies support this postulated topography. [From M. Muekler, C. Caruso, S.A. Baldwin, M. Panico, M. Blench, H.R. Morris, W.J. Allard, G.E. Lienhard, and H.F. Lodish. *Science* 229(1985):941.]

The members of this family have distinctive roles:

1. GLUT1 and 3, present in nearly all mammalian cells, are responsible for basal glucose uptake. Their K_M for glucose is about 1 mM, significantly less than the normal serum glucose level, which typically ranges from 4 mM to 8 mM. Hence, GLUT1 and 3 continually transport glucose at an essentially constant rate.

2. GLUT5, present in the small intestine, works in tandem with the Na^+-glucose symporter in the absorption of glucose from the gut. The symporter pumps glucose into the intestinal epithelial cell. GLUT5 in the plasma membrane on the opposite side of the cell then releases glucose into the bloodstream.

3. GLUT2, present in liver and pancreatic β cells, is distinctive in having a very high K_M for glucose (15–20 mM). Hence, the rate of entry of glucose into these tissues is proportional to the blood glucose level. The pancreas can thereby sense the glucose level and accordingly adjust the rate of insulin secretion. The high K_M of GLUT2 also assures that glucose rapidly enters liver cells only in times of plenty. When the blood glucose level is low, glucose preferentially enters brain and other tissues because their glucose transporters have a lower K_M than that of liver.

4. GLUT4, which has a K_M of 5 mM, mediates the entry of glucose into muscle and fat cells. Insulin, which signals the fed state, leads to a rapid increase in the number of GLUT4 transporters in the plasma membrane. Hence, insulin promotes the uptake of glucose by muscle and fat.

This family of transporters vividly illustrates how isoforms of a single protein can profoundly shape the metabolic character of cells and contribute to their diversity and functional specialization.

SUMMARY

Glycolysis is the set of reactions that converts glucose into pyruvate. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, where most of the free energy in glucose is harvested. The 10 reactions of glycolysis occur in the cytosol. In the first stage, glucose is converted into fructose 1,6-bisphosphate by a phosphorylation, an isomerization, and a second phosphorylation reaction. Two molecules of ATP are consumed per molecule of glucose in these reactions, which are the prelude to the net synthesis of ATP. In the second stage, fructose 1,6-bisphosphate is cleaved by aldolase into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which are readily interconvertible. Glyceraldehyde 3-phosphate is then oxidized and phosphorylated to form 1,3-bisphosphoglycerate, an acyl phosphate with a high phosphoryl transfer potential. 3-Phosphoglycerate is then formed as an ATP is generated. In the last stage of glycolysis, phosphoenolpyruvate, a second intermediate with a high phosphoryl transfer potential, is formed by a phosphoryl shift and a dehydration. Another ATP is generated as phosphoenolpyruvate is converted into pyruvate. There is a net gain of two molecules of ATP in the formation of two molecules of pyruvate from one molecule of glucose.

The electron acceptor in the oxidation of glyceraldehyde 3-phosphate is NAD^+, which must be regenerated for glycolysis to continue. In aerobic organisms, the NADH formed in glycolysis transfers its electrons to O_2 through the electron transport chain, which thereby regenerates NAD^+. Under anaerobic conditions, NAD^+ is regenerated by the reduction of pyruvate to lactate. In some microorganisms, NAD^+ is normally regenerated by the synthesis of lactate or ethanol from pyruvate. These two processes are examples of fermentations.

The glycolytic pathway has a dual role: it degrades glucose to generate ATP, and it provides building blocks for the synthesis of cellular components. The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs. Under physiologic conditions, the reactions of glycolysis are readily reversible except for the ones catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase. Phosphofructokinase, the most important control element in glycolysis, is inhibited by high levels of ATP and citrate, and it is activated by AMP and fructose 2,6-bisphosphate. In liver, this bisphosphate signals that glucose is abundant. Hence, phosphofructokinase is active when either energy or building blocks are needed. Hexokinase is inhibited by glucose 6-phosphate, which accumulates when phosphofructokinase is inactive. Pyruvate kinase, the other control site, is allosterically inhibited by ATP and alanine, and it is activated by fructose 1,6-bisphosphate. Consequently, pyruvate kinase is maximally active when the energy charge is low and glycolytic intermediates accumulate. Pyruvate kinase, like the bifunctional enzyme controlling the level of fructose 2,6-bisphosphate, is regulated by phosphorylation. A low level of glucose in the blood promotes the phosphorylation of liver pyruvate kinase, which diminishes its activity and thereby decreases glucose consumption in the liver.

Induced fit enhances the catalytic efficiency of hexokinase and triosephosphate isomerase by preventing undesired side reactions. The isomerase is an example of a catalytically perfect enzyme limited only by the diffusion-controlled encounter of enzyme and substrate. A thioester intermediate conserves some of the free energy of oxidation of glyceraldehyde 3-phosphate; attack by P_i yields an energy-rich acyl phosphate. Arsenate, an analog of phosphate, uncouples oxidation and phosphorylation.

The thermodynamically downhill entry of glucose into animal cells is mediated by a family of glucose transporters named GLUT1 to 5. The binding site for glucose in this plasma membrane protein, when occupied, alternately faces the inside and outside of the cell. The different K_M values and differential regulation of this family of transporters shape the metabolic character of cells in different organs.

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Where to start

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PROBLEMS

1. *Kitchen chemistry.* Sucrose is commonly used to preserve fruits. Why is glucose not well suited for preserving foods?

2. *Tracing carbon atoms.* Glucose labeled with ^{14}C at C-1 is incubated with the glycolytic enzymes and necessary cofactors.
   (a) What is the distribution of ^{14}C in the pyruvate that is formed? (Assume that the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate is very rapid compared with the subsequent step.)
   (b) If the specific activity of the glucose substrate is 10 mCi/mM, what is the specific activity of the pyruvate that is formed?

3. *Lactic fermentation.* Write a balanced equation for the conversion of glucose into lactate.
   (a) Calculate the standard free-energy change of this reaction using the data given in Table 19-2 (p. 491) and the fact that $\Delta G^{\circ\prime}$ is -6 kcal for the reaction

   $$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{lactate} + \text{NAD}^+$$

   (b) What is the free-energy change ($\Delta G'$, not $\Delta G^{\circ\prime}$) of this reaction when the concentrations of reactants are: glucose, 5 mM; lactate, 0.05 mM; ATP, 2 mM; ADP, 0.2 mM; and P_i, 1 mM?

4. *High potential.* What is the equilibrium ratio of phosphoenolpyruvate to pyruvate under standard conditions when [ATP]/[ADP] = 10?

5. *Hexose-triose equilibrium.* What are the equilibrium concentrations of fructose 1,6-bisphosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate when 1 mM fructose 1,6-bisphosphate is incubated with aldolase under standard conditions?

6. *Double labeling.* 3-Phosphoglycerate labeled uniformly with ^{14}C is incubated with 1,3-BPG labeled with ^{32}P at C-1. What is the radioisotope distribution of the 2,3-BPG that is formed on addition of BPG mutase?

7. *An informative analog.* Xylose has the same structure as glucose except that it has a hydrogen atom at C-6 in place of a hydroxymethyl group. The rate of ATP hydrolysis by hexokinase is markedly enhanced by the addition of xylose. Why?

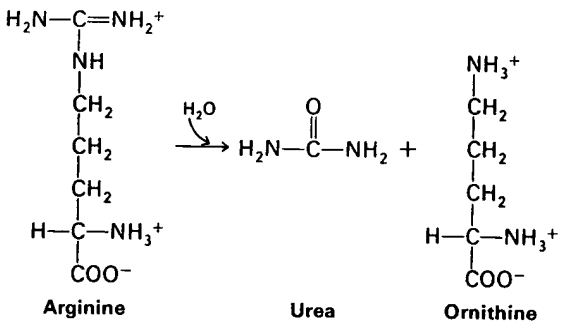
8. *The far reach of glycolysis.* Oxygen transport can be affected in genetic disorders of glycolysis in red cells.
   (a) How are glycolysis and oxygen transport linked?
   (b) How is oxygen affinity altered by a deficiency of hexokinase?
   (c) How is oxygen affinity altered by a deficiency of pyruvate kinase?

9. *Distinctive sugars.* The intravenous infusion of fructose into healthy volunteers leads to a two- to fivefold increase in the level of lactate in the blood, a far greater increase than that observed following the infusion of the same amount of glucose.
   (a) Why is glycolysis more rapid following the infusion of fructose?
   (b) Fructose has been used in place of glucose for intravenous feeding. Why is this use of fructose unwise?

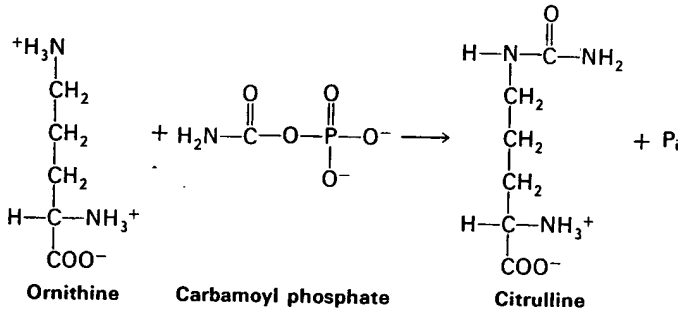
10. *Catalytic metal.* Aldolases in prokaryotes contain a tightly bound divalent metal ion that is essential for catalysis. Propose a catalytic function for this metal ion.

11. *Contrasting inactivators.* Prokaryotic aldolases are inactivated by ethylenediaminetetraacetate (EDTA), a chelator of divalent metal ions, whereas animal aldolases discussed in this chapter are inactivated by sodium borohydride. Account for this difference.

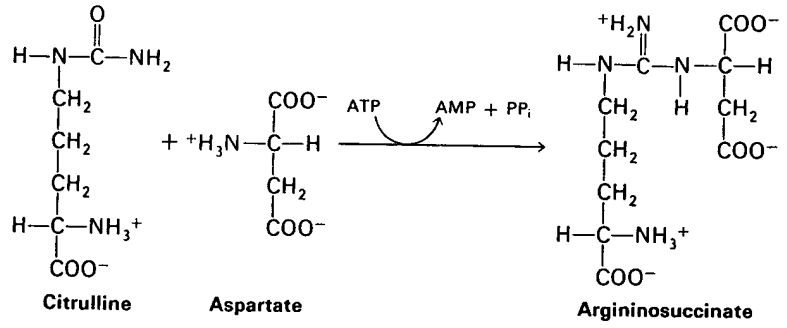
12. *Metabolic mutants.* Predict the effect of each of the following on the pace of glycolysis in liver cells:
   (a) Loss of the allosteric site for ATP in phosphofructokinase.
   (b) Loss of the binding site for citrate in phosphofructokinase.
   (c) Loss of the phosphatase domain of the bifunctional enzyme that controls the level of fructose 2,6-bisphosphate.
   (d) Loss of the binding site for fructose 1,6-bisphosphate in pyruvate kinase.



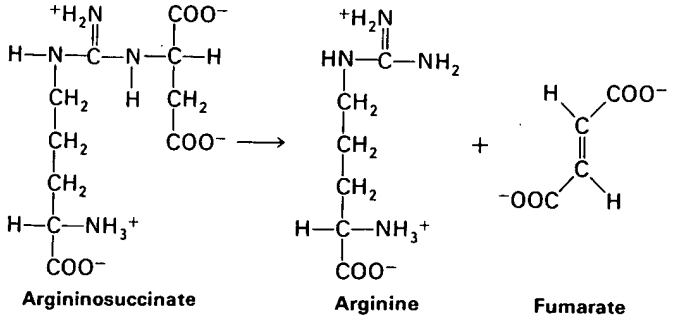
The other reactions of the urea cycle lead to the synthesis of arginine from ornithine. First, a carbamoyl group is transferred to ornithine to form *citrulline,* in a reaction catalyzed by *ornithine transcarbamoylase.* The carbamoyl donor in this reaction is *carbamoyl phosphate,* which has a high transfer potential because of its anhydride bond.



Argininosuccinate synthetase then catalyzes the condensation of citrulline and aspartate. This synthesis of *argininosuccinate* is driven by the cleavage of ATP into AMP and pyrophosphate and by the subsequent hydrolysis of pyrophosphate.



Finally, *argininosuccinase* cleaves argininosuccinate into *arginine* and *fumarate.* These two reactions, which transfer the amino group of aspartate to form arginine, preserve the carbon skeleton of aspartate in the form of fumarate.



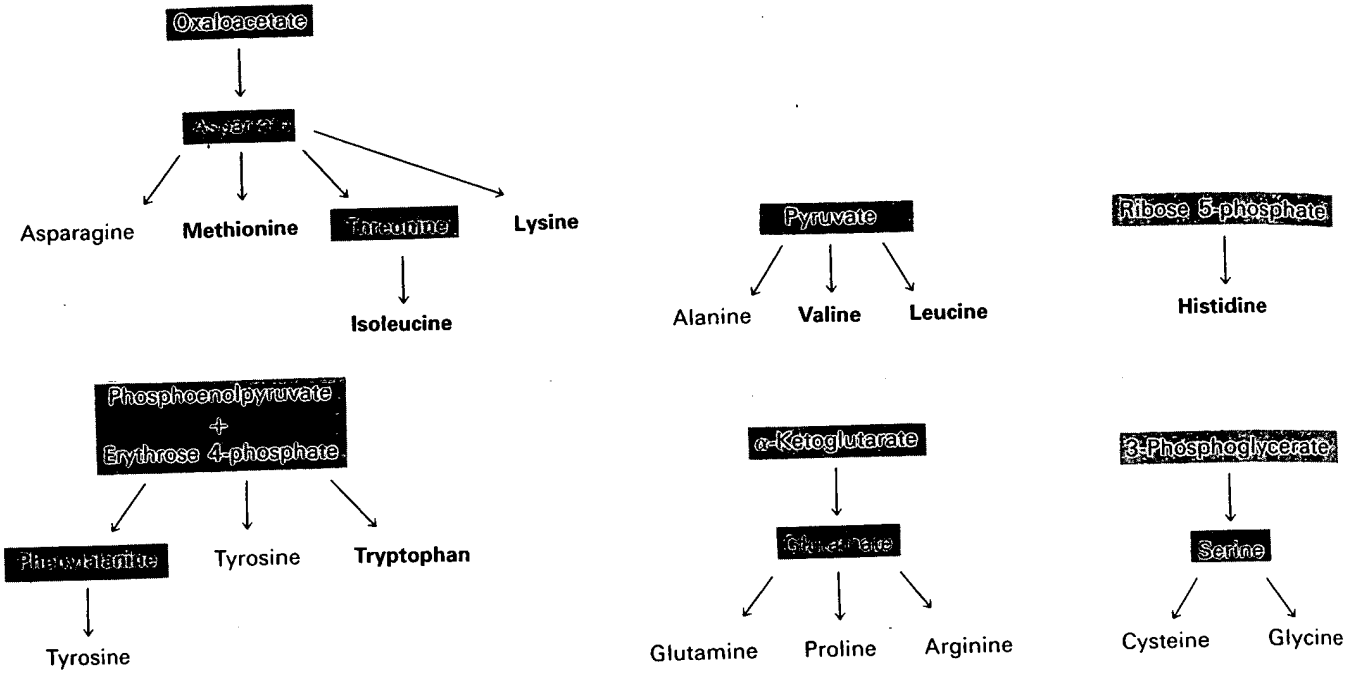


Figure 28-7
Biosynthetic families of amino acids in bacteria and plants. Major metabolic precursors are shaded blue. Amino acids that give rise to other amino acids are shaded red. Essential amino acids are shown in boldface.

The nonessential amino acids are synthesized by quite simple reactions, whereas the pathways for the formation of the essential amino acids are quite complex. For example, the nonessential amino acids *alanine* and *aspartate* are synthesized in a single step from pyruvate and oxaloacetate, respectively. Each acquires its amino group from glutamate in a transamination reaction in which pyridoxal phosphate is the cofactor (p. 631):

$$\text{Pyruvate} + \text{glutamate} \rightleftharpoons \text{alanine} + \alpha\text{-ketoglutarate}$$

$$\text{Oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}$$

Asparagine is then synthesized by the amidation of aspartate:

$$\text{Aspartate} + NH_4^+ + \text{ATP} \longrightarrow \text{asparagine} + \text{AMP} + PP_i + H^+$$

In mammals, the nitrogen donor in the synthesis of asparagine is glutamine rather than NH_4^+, as in bacteria. Ammonia generated at the active site of the enzyme is directly transferred to bound aspartate. Recall that high levels of NH_4^+ are toxic to humans (p. 637).

Another one-step synthesis of a nonessential amino acid in mammals is the hydroxylation of phenylalanine (an essential amino acid) to *tyrosine.*

$$\text{Phenylalanine} + O_2 + \text{NADPH} + H^+ \longrightarrow \text{tyrosine} + NADP^+ + H_2O$$

This reaction is catalyzed by *phenylalanine hydroxylase,* a monooxygenase discussed previously (p. 647). It is noteworthy that tyrosine is an essential amino acid in people lacking this enzyme.

GLUTAMATE IS THE PRECURSOR OF GLUTAMINE, PROLINE, AND ARGININE

The synthesis of glutamate by the reductive amination of α-ketoglutarate has already been discussed, as has the conversion of glutamate into *glutamine* (p. 717). Glutamate is the precursor of two other nonessential amino acids, *proline* and *arginine.* First, the γ-carboxyl group of glutamate reacts